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EXPERIMENTAL STUDIES OF NATURAL PURIFICATION IN POLLUTED WATERS

X. REOXYGENATION OF POLLUTED WATERS BY MICROSCOPIC ALGAE

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FOREWORD

Previous papers (1, 2, 3, 4) on the general subject "Experimental Studies of Natural Purification in Polluted Waters" have dealt successively with apparatus and technique, a suitable reproducible medium, extent of bacterial growth in different concentrations of medium, and, finally, the plankton as a factor concerned in the oxidation of organic matter.

Keeping in mind the general purpose stated in the initial paper, "to acquire more extensive and exact knowledge concerning the operation of natural agencies in the process of purification of sewagepolluted water", and confronted with the highly complex situation which invariably prevails in a natural stream (after prolonged and intensive study of the Potomac, the Ohio, and the Illinois Rivers), it has seemed necessary to conduct our laboratory studies under conditions of such control as would make possible a reasonable interpretation. The start was made by adding a single kind of bacteria, in pure culture, to the selected medium, and interpreting results as expressed by the dissolved oxygen history during a period Then, a plankton organism—a bacteria-eating protozoan was added and the experiments were repeated, with significant variations in the dissolved oxygen history of these cultures, as compared with the similar history of the cultures which contained bacteria This protozoan was in pure culture, and bacteria-free, hence resulting differences in the dissolved oxygen picture of the cultures could be ascribed to no other agency than the protozoan itself.

These experiments were repeated, but in none of the cultures were living plants introduced, a matter of difficulty because of the necessity of sufficient sunlight to enable the plants to function. Meantime, the matter seemed to be one of such importance that it could not be disregarded, inasmuch as a natural water body always contains not

only bacteria and protozoa, but green plants also, of microscopic size or larger. Seeking information that would be serviceable in an understanding of the interrelations of the various factors usually found in natural streams, we were forced to the conclusion that the activities of minute plants had the same claim for attention as did the activities of bacteria and protozoa. Our work was incomplete without a study of aquatic plants and an attempted appraisal of their unique activity.

We used the same medium and the same bacterium in pure culture as were used in the previous experiments. However, instead of bacteria-eating protozoa, we used a pure culture of unicellular green alga, which, like all chlorophyll-bearing algae, gives off excess oxygen in the presence of sunlight. Thus there was projected into the picture as a factor the unique activity of green plants producing in situ a quantity of the very material, dissolved oxygen, by which the net efficiency of the several interrelated activities was to be measured. Atmospheric oxygen was meantime excluded from the cultures.

This paper presents the results of a series of controlled experiments carried out in an effort to learn whether certain minute chlorophyll-bearing plants common in natural water are able, if present in moderate number, to provide a measurable and significant quantity of oxygen, if exposed to such amounts of sunlight as normally occur from day to day and if unaided and unaffected by atmospheric aeration. We also attempt a tentative and approximate measure of the amount of such oxygen in comparison with the approximate volume of the plants which have produced it.¹

RELATED STUDIES

In 1911 there was published an authoritative study (5) of dissolved gases in Wisconsin lakes, supported by basic data of unquestioned quality in convincing amount. Much emphasis was placed on the free CO₂, and also on the half-bound CO₂ contained in dissolved bicarbonates, the alkaline waters thus formed supporting the richest growth of algae, and consequently the heavier growth as well, of such microscopic animals as were dependent on the algal growths for food. During calm, clear weather, positive correlation was frequently indicated between abundance of algae and high content of dissolved oxygen. Apparently the only available explanation was the photosynthetic activity of the algae.

In 1912 Chambers' study (6) of the relation of algae to dissolved oxygen and carbon dioxide was published. Chambers summarizes, in part, as follows:

¹ It is desired to acknowledge the faithful and efficient cooperation, throughout these experiments, of Junior Bacteriologist Orena B. Stewart, who prepared the media, did practically all of the bacteriological work, and made the oxygen determinations. Also, the successful use of the finger-cot seal was due largely to Mrs. Stewart's skill.

"There is an intimate and mutual relation between the algae and submerged aquatics in a body of water and the gases dissolved in that water. They fluctuate together.

"Air or its constituents, oxygen and CO₂, are as essential to water plants as water is to land plants, and equally difficult to secure.

"The photosynthesis of rapidly-growing algae and aquatic plants in a body of water may diminish or deplete the supply of CO₂ and increase the oxygen content beyond saturation."

In 1913 the writer studied the extensive plant-filled shallow areas forming expansions of the Potomac River (7) and showed that the great masses of submerged plants function as oxygenators of the sewage-polluted water. That such increase in oxygen content was not due to atmospheric aeration incident to the spreading out of the water on the flat was repeatedly indicated when samples which had been exposed to plant activity on the flats during daylight averaged 91.5 percent saturation as compared with 82 percent saturation averaged by samples which had been on this same plant-filled flat during hours of darkness. Incidentally, it was emphasized that these samples collected in the afternoon showed a very different dissolved oxygen status of the water as compared with samples collected in the forenoon. The dissolved oxygen content of the water averaged 103 percent saturation on bright, sunny days, as compared with an average of 75 percent of saturation in samples collected at the same place on dark, The plants concerned were not only the larger, easily cloudy days. visible kinds (eel-grass, filamentous algae, and the like), but also innumerable microscopic forms, including diatoms.

Butterfield (2), seeking to develop a suitable synthetic medium with which to study the dissolved oxygen history of cultures of bacteria only, and again of bacteria and protozoa (both in pure culture) states that, in preliminary work, (1) bacteria increased rapidly in numbers, (2) to a rather definite limit, and (3) depletion of dissolved oxygen occurred meanwhile at the usual, well-defined rate. In further carefully checked cultures of B. aerogenes only, the bacterial content tended to remain constant after reaching their limiting numbers, and, at this point, oxygen depletion practically ceased; but in exactly similar cultures which, however, contained also the bacteria-eating protozoan Colpidium, (1) a rapid bacterial decline occurred, the bacteria apparently being consumed by the protozoa, (2) these greatly increased meantime, and (3) depletion of dissolved oxygen continued without interruption.

Mohlman and associates (8), studying data obtained from the sewage-polluted Illinois River at hourly intervals for a year, state that "in a highly polluted stream an hourly variation in concentration may occur similar to the well-known variation in concentration of sewage throughout the 24 hours. In the lower reaches of the same

stream, the same, if not greater, variation may be found in the dissolved oxygen content due to the presence of green and blue-green algae which give off large amounts of oxygen in the presence of sunlight."

In discussing the observed differences and variations, the authors point out the necessity of caution in accepting a single daily sample at a given station as representing the average conditions.

Rudolfs and Heukelekian in a study (9), whose purpose was the evaluating of the role of green organisms in the reaeration of the Delaware River, found rapid increase of dissolved oxygen during the morning hours, reaching maximum in the afternoon and declining thereafter and during the hours of darkness until a minimum was reached about the time of sunrise. The authors state that "in dealing with the pollution of a stream the role of reaeration by green organisms must be properly evaluated * * * because the temporary condition in the afternoon is by no means the daily average condition."

Birge and Juday (11), studying the penetration of light, found that in most transparent lakes 1 to 4 percent of the solar energy which was delivered at the surface penetrated to 18 meters, and a large growth of the moss *Drepanocladus* was found at this depth. In a highly-colored lake, however, light penetration was much less, being reduced to 0.5 percent at a depth of only 2 meters.

The writer, in a brief restudy of the Potomac River in 1932 (12), with a view to finding out whether the great areas of submerged plants were still as effective in producing oxygen as in 1914, found average percentages of saturation of dissolved oxygen as follows:

Station	Average p uration o oxygen	ercent sat- of dissolved
	1914	1932
Water of river channel Water of plant-filled flat. Water of plant-filled flat on cloudy days. Water of plant-filled flat on sunny days. Water ebbing to river channel after night hours on the plant-filled flat (morning samples). The same after 4 to 6 daylight hours on the same flat (afternoon samples).	71 87 75 103 82 92	51 80 81 108 59

Inspection of the averages indicates the wide variation to be expected in dissolved oxygen content in a plant-affected environment, (1) on cloudy or on sunny days and (2) in samples showing plant effects during daylight hours as compared with night hours. Obviously, the hour of collecting samples is highly important when averages show a possible difference of from 10 to 34 percent in samples collected in the morning and the afternoon, respectively, as in the last two items in the tabulation.

In 1932, Olson (13), studying Minnesota lakes and taking samples 8 feet under the surface, found that late in the afternoon the water showed 218 percent saturation, apparently due to photosynthetic oxygen produced by Aphanizomenon, but at 3 o'clock in the morning only 48 percent saturation was present. Meantime, samples taken at the same place, but 18 feet under the surface, showed only 38 percent saturation in the afternoon and the same at 3 a.m. Obviously the oxygen fluctuation due to plant activity was confined to the surface stratum of waters which light could penetrate, for at 18 feet "dissolved oxygen was as low after a long sunny day as it was after a dark, moonless night."

Olson studied a shallow lake which contained large amounts of macroscopic submerged vegetation. This lake was also polluted by sewage and creamery wastes. Desiring reliable data as to the net effect, on the lake water, of these two antagonistic agencies, the one supplying oxygen during hours of sunlight, the other using up dissolved oxygen all the time, day and night, Olson collected hourly samples for dissolved oxygen determination at four representative points in this lake, with results as follows:

Table O.—Variation in dissolved oxygen during a 24-hour period (Olson) [Clearwater Lake, Waconia, Minn., July 22-23, 1932]

	8	Station .	A	8	Station B			Station C			Station D		
Time	D. 0.	Tem- pera- ture ° C.	Per- cent satu- ration	D. 0.	Tem- pera- ture ° C.	Per- cent satu- ration	D. 0.	Tem- pera- ture ° C.	Per- cent satu- ration	D. 0.	Tem- pera- ture ° C.	Per- cent satu- ration	
10 a. m	3.9 4.52 5.6 5.11 9.3 10.5 11.1 9.3 11.6 5.5 4.7 2.1 3.7 4.7 2.7 4.7 2.7 4.7 2.7 4.7 2.7 4.7 2.7 4.7 4.7 4.7 4.7 4.7 4.7 4.7 4.7 4.7 4	25 26 27 29 29 28 26 26 25 25 25 24 24 24 25 26	36, 9 47, 4 54, 7 63, 2 81, 7 65, 6 65, 6 119, 7 136, 3 130 141, 1 135, 2 109, 8 134, 8 90, 7 77, 5 62, 1 53, 9 43, 4 24, 6 35, 8 32, 8	7 10. 5 6 1 14. 6 1 14. 1 14. 2 11. 1 1 14. 2 11. 5 8 8 4 9 5 5 9 3 7 2 2 0 3 8 8 3 8 8 3 8 8	26 26 27 28 28 28 26 25 25 24 24 24 24 24 24 24 24 24 24 24 24 24	85. 1 127. 7 226. 2 122. 9 190. 6 140. 1 165. 4 179. 3 172. 7 148. 4 137. 2 116. 9 95. 4 43. 4 25. 8 35. 1 44. 5 42. 2	12. 8 19. 2 20. 3 21. 9 23. 3 22. 5 23. 5 23. 5 21. 7 20. 3 15. 7 12. 4 8. 6 9 5. 7 6. 9 8. 2 8. 2	86688888888888888888888888888888888888	155. 7 167. 9 233. 6 246. 9 276. 5 294. 1 300. 8 296. 7 294. 2 264. 9 1137. 3 147. 9 102. 6 97. 8 91. 4 93. 8 93. 8 91. 4 93. 6 65. 6 96. 1 128. 9	11. 3 12. 9 14. 0 15. 1 15. 1 16. 3 16. 3 16. 3 15. 7 14. 8 12. 8 11. 9 10. 0 8. 9 10. 0 8. 9 9. 9	24 26 27 28 28 28 27 25 26 25 24 24 24 24 24 25 25 25 25 25 25 25 25 25 25 25 25 25	132. 5 132. 5 156. 9 170. 3 187. 1 190. 6 200. 8 205. 8 205. 8 194. 5 163. 5 170. 3 167. 0 110. 2 127. 7 142. 0 110. 2 127. 7 124. 3 144. 2 96. 1 109. 8 118. 1	

Location of stations:

Station A: Near the creamery sewer outlet.

Station B: 500 feet west of station A. (Submerged plants abundant.)

Station C: 500 feet northwest of station B. (Filamentous algae abundant.)

Station D: 500 feet northeast of station C. (No visible pollution. No visible plants.)

Thus the actual results obtained by these 24-hour samples indicate, in brief, that at station A, in the presence of heavy pollution from a creamery and of an abundant growth of submerged plants, the forces of aeration and of deaeration were apparently quite evenly matched, supersaturation reaching a low maximum of only 141.1 percent. Without plant-made oxygen stored during the day, night time might have shown a condition of near or actual depletion in this polluted area.

At station B, removed somewhat from heavy pollution but having, on the other hand, a thick bed of submerged plants, aeration was more than a match for deaeration, not only in frequency but also in magnitude, supersaturation reaching a high maximum of 236.1 percent.

At station C, still farther away from the creamery outfall, and in the presence of filamentous algae in large amounts, the dissolved oxygen content dropped below saturation only 8 times, and a maximum of 300.8 percent saturation was attained.

At station D, with neither visible plants nor polluting wastes near, the water showed only a single instance of less than saturation.

Calvert (14) studied the White River below the Indianapolis sewage plant outfall. The outstanding results of the study were as follows:

(1) Numerous samples collected in the morning, and again in the afternoon, showed the effects of plant activity in the consistently larger amounts of dissolved oxygen in the afternoon samples.

(2) Samples collected on a cloudy, rainy day showed less dissolved oxygen in the afternoon than in the forenoon, due apparently to decomposition without the compensating effect of photosynthesis,

which requires sunlight.

(3) When the very high chloride content indicated an unusual amount of organic matter, neither morning nor afternoon samples showed dissolved oxygen. All were negative. Apparently the organic load was more than a match for the combined aerating effect from the atmosphere and from photosynthetic activity respectively.

Calvert further points out that dilution, time of day when samples are taken, and weather conditions may so affect the dissolved oxygen content that the indicated condition of the water is by no means the true or average condition.

Hubbs (15), discussing the many interrelated factors requiring consideration in any appraisal of the supposed damage (or benefit) done to fish life by sewage, points out that a badly polluted stream may show saturation, or even supersaturation, with dissolved oxygen (due to plant activity), especially if the samples be collected on sunny afternoons; but this same stream may, during the night and toward morning, show complete depletion of dissolved oxygen. It is thus shown that the oxygen content as indicated by the usual samples conveniently collected during the daytime does not give adequate information.

Schomer (16), investigating photosynthetic activity of certain water plants in Wisconsin lakes, found optimum conditions for photosynthesis varying (1) with weather conditions, sunny days being more effective than cloudy, (2) with depth as related to amount of sunlight available to the plants, and (3) with the kind of plant used. The greatest photosynthetic activity was found to be from 10:30 a. m. to 1:30 p. m.

THE PRESENT STUDY

GENERAL ITEMS OF SET-UP

In an attempt to simulate natural conditions so far as possible and yet maintain adequate laboratory control, the experiments were carried out as follows: (I) In sunlight, without motion of bottle contents; (II) in sunlight, with continuous motion of bottle contents; (III) in darkness, without motion; (IV) in sunlight, with motion of contents but in bottles stoppered with cotton plugs, thus affording contact with external air, and actual circulation of air. In all cases, bottles of 300-cc capacity, with ground-glass stoppers, were used; but in series IV these stoppers were replaced with cotton plugs.

In I, the sunlight, without motion experiment, the bottles were merely set upright on a shelf in a south window, in which position the cultures received direct sunlight, on a sunny day, for about 5 hours. There were usually 16 bottles in a set, with 2 or 3 extras, such as those on the shelf.

In II sunlight, with motion of bottle contents (causing continuous mixing), the 16 bottles were uniformly spaced and anchored in an inclined position within a horizontally rotating cage constructed of laths, which were spaced to admit sunlight to the culture. The cage, 1 foot by 1 foot by 3 feet, was placed horizontally on a shelf in a south window and rotated on its long axis about four times per minute.

In series III, without motion and without light, the bottles were placed in the 20° C. incubator. The outstanding factor in this case was the constant temperature.

In series IV the culture bottles were arranged on a pair of narrow hanging shelves, the weight of each shelf being counterbalanced by the weight of the other one. This contrivance, with its supporting framework, is referred to as the "elevator."

The bottles were placed in this elevator in pairs, one bottle on the front shelf, its companion bottle on the back, or second, shelf. Two bottles constituted one sample, the two being connected by an overhead siphon made in part of rubber tubing to provide flexibility. Into the two 300-cc bottles a total of 500 cc of medium was placed, thus leaving space within the bottles of a given pair for such medium as would be siphoned from one bottle to the other as the two shelves changed position in response to power applied periodically by a motor.

These shelves shifted once in 6 minutes. Every shift, changing the relative level of the two bottles of any given culture by about 2 inches, caused the transfer, by overhead siphon, of about 80 cc of the total 500 cc (16 percent) in the two bottles. Actual flow between the two bottles required a minute or less.

These pairs of bottles in the elevator were stoppered with cotton plugs through which the glass ends of the siphon were passed. In addition, a mat of sterilized cotton 1 inch thick, wide enough to envelop the entire bottle neck and to project about an inch or more above it, was wrapped around the bottle top and tied at top and bottom. External air gaining access to the bottle contents had to pass through this mass of sterilized cotton.

Cultures in the elevator were exposed in an east window and received direct sunlight in the morning only, until about 9 a. m., and diffused light and "sky-shine" the remainder of the day. This experiment was carried out in July.

Temperature.—In all the experiments carried out in the presence of sunlight, temperature was a factor incapable of control. Cultures motionless on the shelf exposed to direct sunlight would attain a temperature higher by two or three degrees than those cultures on the same shelf which were partly protected from direct light.

In the absence of temperature control, as in a window exposed (1) to direct sunlight daily for 5 or 6 hours, or (2) to cloudy conditions when these occur, or (3) to complete absence of sunlight during the night, there is inevitable fluctuation—especially the day and night fluctuations, which produce slight changes (expansion or contraction) of the bottle contents. Rise in temperature must necessarily force out, from a completely filled bottle, a small amount of the contained liquid. If this be water or similar liquid, it very soon evaporates, unless the external air with which it comes in contact be saturated, a very unusual condition.

Similarly, falling temperature, causing slight contraction of bottle contents, will produce a condition of partial vacuum within the bottle which must be relieved at the only point where this is possible, viz, about the periphery of the ground-glass stopper, the possible and probable result being a small body of air "sucked" into the bottle and forming a bubble at the lowermost end of the ground-glass stopper. It is also possible that a bubble may be formed within the bottle when, on rise of temperature, the dissolved gases already present must escape to some degree.

In dissolved oxygen determinations as usually performed in a laboratory, the danger of inaccuracy due to the presence of a bubble has been met (1) by an expanded bottle neck of such shape that a protective collar of water surrounds the stopper; (2) by inverting the bottles in a pan of water; (3) by storage in an incubator, thus eliminating tem-

perature fluctuations. In a series of experiments set forth in this paper, exposure to sunlight was necessary, and resulting fluctuations of temperature became a factor with which we were obliged to deal as best we might. Conditions of motion, as in the cage and the elevator, ruled out the use of a bottle with expanded neck and protecting water collar, and also prohibited recourse to the inverting of bottles in a pan of water.

In three experiments (numbers 1, 2, and 3) we had small bubbles of uncertain origin in many of the bottles Recognizing this condition as incompatible with desired accuracy of the results, we sought to correct, or to minimize, this possible hazard. These efforts resulted in the "finger-cot seal" device.

The finger-cot seal.—Finger cots of the largest size obtainable should be used. They must be fresh stock.

The mushroom-top glass stoppers, high form, are necessary in order that the finger-cot, when in position, may be sharply divided into two compartments, above and below this flat, circular mushroom-top, respectively. This portion of the glass stopper should be gone over previously with a file. The rim of the bottle neck must be free from nicks or rough places.

Bottles and their stoppers should be numbered so that stoppers do not become mixed. For sterilizing, cover the unstoppered bottle with a paper cap, tied loosely. Wrap each stopper in a small square of paper, folding or closing the edges of the paper above the flat mushroom-top. Mark, in the paper of the wrapped stopper, the number of the bottle to which this stopper belongs.

Open up the finger-cots so water will freely enter each one. Place all in a large beaker, two-thirds full of water, for sterilization in the autoclave, tying a paper cap over the beaker.

Have an assistant, wearing sterile rubber gloves, grasp, with thumb and forefinger of each hand, opposite sides of the open end of a sterilized cot, lifting it, nearly filled with the sterile water, from the beaker, and holding it firmly.

With the right hand grasp the wrapped stopper, and, holding it by the lowermost end (which is still wrapped in paper), with left hand separate widely the folds over the mushroom top, so as to expose this top, but without touching the glass with the fingers.

With the right hand crowd this exposed glass top down into the sterile finger cot, which the assistant holds firmly meantime and stretches the open end from side to side in order to admit the flat circular top of the glass stopper. This top should now be crowded down at least an inch into the finger cot. Now remove the right hand from the stopper, bringing away meantime the paper wrapping.

Quickly remove the paper cap from the top of the filled bottle which is about to receive the stopper, and steady the bottle firmly while the assistant, retaining with both hands his original thumb-and-finger hold on the cot (now containing the inverted stopper), inserts the stopper into the bottle and with the same movement "snaps" the rubber finger cot down around the neck of the bottle.

We now have the filled culture bottle with the stopper inserted, without bubble, and without having been touched except by sterile gloves. The sterile finger cot, superimposed over the stopper and pulled down around the bottle neck, shows two compartments: (1) a nipple-like projection above the top of the stopper, and (2) an enclosed space, roughly spool-shaped, between the under surface of the stopper top and the uppermost part of the bottle neck. The vertical dimension

of this enclosed space is about one-half inch. Compartment 1 contains water and this water is sterile; compartment 2 contains chiefly air.

Press the stopper firmly into place if necessary. Then with dry finger and thumb pinch and take hold of the rubber at a point on the edge of the mushroom top and gently but firmly pull the rubber away from contact with this edge, making a passageway between compartments 1 and 2. Meantime tip the bottle slightly in the opposite direction. The air in compartment 2 will now pass up into compartment 1, displacing the water, which will meantime flow down into compartment 2, completely filling this space and forming a collar of sterile water enclosing the periphery of the ground-glass stopper.

The walls of this finger-cot seal are elastic, and the culture bottle equipped with it may be turned over and over (as in the rotating cage) without lessening the effectiveness of the seal. This seal will prevent access of outside air to the bottle contents, and if, with fluctuating temperatures, a concentration of bottle contents tends to replace a partial vacuum thereby created, the only available material for this replacement is the sterile water held in place by elastic walls and surrounding the stopper at the point where this enters the neck of the bottle. No known device will absolutely prevent the escape of excess oxygen (or other gas) from the bottle.

THE CULTURES

Contents of culture bottles.—Three classes of cultures were used, as follows: (1) Bacteria only; (2) bacteria and alga; (3) alga only. In the first three experiments, the alga-only cultures were omitted.

The alga used was a bacteria-free culture of *Oöcystis*, isolated by dilutions of polluted river water plated in dilute agar and the resulting isolated colonies picked and thus transferred to broth.

Occystis is thus described by Needham and Lloyd (17): "The ellipsoidal cells exist singly or a few are loosely associated together in a clump of mucus. The cells possess a firm smooth wall which commonly shows a nodular thickening at each pole."

In the more recent text by Smith (18), further details are mentioned: "The cells are broadly to narrowly ellipsoidal * * * and with rounded to somewhat pointed poles. The cell wall is thin and without spines or other ornamentation except for a small nodular thickening at each pole * * *. Sixteen species have been recorded as occurring in the United States."

I believe the species used in our experiments to be *Oōcystis lacustris*. The poles are somewhat pointed, and the cells are 7 to 10 or more microns long.

The bacteria used was a suspension of Bacterium aerogenes in pure culture.

The medium was double strength "synthetic sewage" devised by Butterfield (1929), (2, 3). This is—

Distilled water, buffered at pH 7.2 with phosphate salts in concentration of 2.5 grams per liter. Dextrose-peptone-phosphate broth, per liter 2 cc.

The above represents a concentration of 10 milligrams of dextrose and of peptone per liter.

This stronger medium was selected in consideration of the interrelated facts that limited capacity of the cage (16 bottles) enabled us to examine only 5 sets (of 3 bottles each) after the initial examination. These examinations were so spaced that the total interval covered was from 10 to 17 days. In order to obtain sufficient response from the slow-growing plants, as well as from the rapidly growing bacteria, not only this interval of time, but also the stronger medium, was considered necessary.

This medium was sterilized in two or three carboys. When entirely cooled, a suspension of B. aerogenes was added to two carbovs; to one of these two was added a suspension of the alga Oöcystis in measured amount. To a third carboy was added only the alga sus-Since this alga was growing in broth (which would add materially to the available dissolved organic matter in the carboy), a like amount of the alga suspension, after being killed by heat, was added to the carboy containing bacteria only. This was done in order to equalize the available food in the two carboys containing bacteria. All carboys were vigorously shaken for several minutes to mix and aerate the contents and to bring all cultures to the same basis at the After carboys had stood quiet for 30 minutes, the contents were siphoned, with aseptic precautions throughout, into the numbered 300-cc bottles, which were then placed in their respective positions in the cage, the incubator, the elevator, or on the shelf. Initial examinations were made for the content of bacteria, of alga, and of dissolved oxygen. The pH was uniformly 7.1, and remained at or very near this point throughout the experiment.

A "log" was kept of each experiment, particularly of weather conditions and the approximate number of hours of sunshine, of "skyshine", and abundance and kind of clouds, including partial or total "overcast." The uncertain sequence of dark days and sunny days is one of the major conditions affecting plant life in nature, and the plant-work done under such natural weather conditions is a reasonable measure of what we may expect in the average watercourse.

We have insufficient data relative to large volumes of smoke from railroad yards about 150 yards to the south of the southern-exposure window housing our cultures. This smoke was the more effective on still days. Wind became a factor by quickly scattering the smokepall on certain days. Lack of adequate data relating to these local clouds obviously decreases the reliability of our weather record.

The light concerned in the following experiments passed through panes of ordinary window glass and also through the cylindrical wall of flint glass constituting the culture bottle before reaching the contained organisms. No attempt was made to measure the limitations thus imposed, as compared with the natural, unobstructed light avail-

able to plants in a natural water body. All examinations were made about 1 p. m.

In all cases the bacterial counts were made from agar plates after 24 hours' incubation at 37° C. The dissolved oxygen determinations were made by the usual Winkler method. The counts of alga cells were made by the use of a Sedgwick-Rafter counting cell. The alga cells were of such size that about 50 were required to make up a volume equal to one cubic standard unit (a cube with an edge of 20 microns). This count of alga cells was, therefore, simply the actual number of cells present divided by 50, and further expressed in parts per million by volume by dividing the number of cubic standard units by 125 (19).

Experiments 1 to 4, inclusive, were carried out in 1931, and experiment 5 was carried out in July 1932.

THE INDIVIDUAL EXPERIMENTS

Experiment No. 1.—January 27 to February 6, 1931.

Cultures were of two kinds, viz, B. aerogenes only, and B. aerogenes plus the alga Oōcystis. Both cultures were exposed to light, without motion, and duplicates were exposed to sunlight, with motion meantime. The pH remained at about 7.1 throughout. In the 10.5 days' duration of the experiment, there were 12 sunny half-days and 9 cloudy half-days. The hours of daylight, both cloudy and sunny, made up about 33 percent of the total time, and the sunny hours alone formed 19 percent of the total of 252 hours. Data are recorded in tables 1 and 2.

Table 1.—History of dissolved oxygen in bacterial cultures with and without alga, with no atmospheric aeration meantime. In sunlight, and without motion

		Bacter	ria only	Bacteria and alga			
Days	Temper- ature °C.	Bacteria in 1 cc (in thousands)	D. O., p. p. m.	Bacteria in 1 cc (in thousands)	D. O., p. p. m.	Alga in 1 cc (in p. p. m. by vol- ume)	
(1)	(2)	(3)	(4)	(5)	(6)	(7)	
0	23 23. 5 22. 5 25. 5 23 23. 5 29. 5	7. 4 163 3, 740 3, 070 3, 690 3, 650 2, 690 430	7. 71 7. 95 5. 32 5. 25 5. 34 6. 00 6. 68 5. 58	6. 6 610 2, 790 3, 770 4, 700 2, 660 1, 280 1, 080	7. 80 6. 20 5. 52 6. 00 6. 66 8. 00 12. 02 12. 34	1. 28 1. 44 2. 24 2. 24 5. 40 17. 70 37. 10 54, 70	

Table 2.—History of dissolved oxygen in bacterial cultures with and without alga, with no atmospheric aeration meantime. In sunlight, and with continuous motion

		Bacter	ia only	Bacteria and alga			
Days	Temper- ature °C.	Bacteria in 1 cc (in thousands)	D. O., p.p. m.	Bacteria in 1 cc (in thousands)	D. O., p. p. m.	Alga in 1 ce (in p. p. m. by vol- ume)	
(1)	(2)	(3)	(4) (5)		(6)	(7)	
0	23 23 23 27 24. 5 23. 5 29. 5	7. 4 301 6, 100 3, 920 3, 900 3, 290 3, 280 1, 960	7. 71 6. 50 4. 85 5. 20 4. 60 4. 53 5. 70 5. 24	6. 6 112 2, 430 3, 370 5, 700 2, 490 1, 640 1, 420	7. 80 7. 35 5. 05 5. 42 5. 38 8. 30 9. 12	1. 28 2. 40 1. 60 2. 88 4. 25 10. 80 35. 80 69. 80	

Particular attention is invited to the great increase in alga content (column 7), and also to the increase in dissolved oxygen content meantime (column 6), notwithstanding the consumption of some oxygen by the growth of bacteria. Inasmuch as the bacterial content and growth are approximately the same as in the bacteria-only culture run at the same time and under the same conditions (columns 3 and 5), we may reasonably assume that the dissolved oxygen required by bacterial growth in each case is about equal, or approximately 2.13 parts.

The significant feature in the behavior of the bacteria-only cultures lies in the fact that oxygen is consumed but is not replaced. The equally significant fact shown by the bacteria-plus-alga cultures is the replacement of oxygen. In this particular experiment the proportion of sunshine (19 percent) has made possible such plant increase during 10 days that the oxygen thereby produced meantime far more than equals the amount used by the bacterial content, which, at the maximum, numbers nearly 5 million per cc. Less sunshine would logically mean less plant growth and less oxygen.

The dissolved oxygen record in table 2 is very similar to that given in table 1. Decrease in content is correlated with initial increase in bacterial numbers, and there is no marked and sustained tendency thereafter for this lessened oxygen content to recover by replacement of the consumed oxygen.

Again we note, as in table 1, that the outstanding difference between the bacteria-only cultures and the bacteria-plus-alga cultures lies in the ability in the latter to replace the oxygen used by bacterial growth. Thus far this replacement efficiency has been more than a match for the consumption factor. This fact is indicated by the presence, after 10 days, of greater amounts of dissolved oxygen than were present at the start.

Summary.—1. The addition of motion (continuous mixing of the bottle contents) apparently makes no great difference in bacteria,

alga, or dissolved oxygen content. However, the growth of both bacteria and alga is somewhat the greater during the last 4 days, in those cultures having motion.

- 2. Consumption of dissolved oxygen is correlated, both as to time and magnitude, with the increase in bacterial growth, in both the bacteria-only experiments.
- 3. In the similar bacterial history of each of the experiments where the cultures contained both bacteria and alga, the dissolved oxygen shows a drop from the initial, then gradual, but very marked recovery and further increase above the initial content.
- 4. Growth of the alga in these same cultures, in both experiments, showed a heavy increase over the initial content, especially during the final 4 days, when the dissolved oxygen also showed increase above the initial content.

Experiment no. 2.—February 9 to February 20, 1931, inclusive.

During the 11 days of the experiment, daily weather observations showed a preponderance of cloudy days, there being 15% cloudy half-days (62 hours) and 6% sunny half-days (26 hours), as compared with the 9 cloudy half-days and 12 sunny half-days of experiment no. 1. This is on the basis of 8 hours of effective sunlight each day.

On this same basis there are 16 hours of essential darkness each day, or a total of 176 hours of the total 264 hours' duration of the experiment. Thus during 65 percent of the time there was no possibility of any photosynthesis. During the remaining 35 percent of the time, a large percentage of dark and cloudy days reduced the time during which active photosynthesis was possible to 26 hours, which is only 10 percent of the total duration of the experiment. On a basis of effective photosynthesis hours, in the previous study (experiment no. 1) there were almost twice as many light hours as were available to experiment no. 2.

Results of experiment no. 2 are recorded in tables 3, 4, and 5.

Table 3.—History of dissolved oxygen in bacterial cultures with and without alga, with no atmospheric aeration meantime. In sunlight, and without motion

		Bacter	ia only	Bacteria and alga			
Days	Temper- ature ° C.	Bacteria in 1 cc (in thousands)	D. O., p. p. m.	Bacteria in 1 cc (in thousands)	D. O., p. p. m.	Alga in 1 co (in p. p. m. by vol- ume)	
(1)	(2)	(3)	(4)	(5)	(6)	(7)	
0	20. 5 18. 5 20. 5 19. 5 19 20 19 20	8. 6 410 5, 900 8, 650 6, 600 6, 700 5, 000 690 3, 590	8. 45 7. 90 6. 38 5. 54 5. 78 5. 68 5. 68 6. 68	8. 5 43 8, 050 8, 800 7, 350 6, 800 1, 680 2, 750 3, 700	8. 30 8. 30 5. 72 5. 82 6. 22 6. 12 6. 62 7. 08 8. 02	2. 56 3. 84 4. 32 4. 32 5. 45 5. 45 8. 00 9. 60 13, 76	

Table 4.—History of dissolved oxygen in bacterial cultures with and without alga, with no atmospheric aeration meantime. In sunlight, and with continuous motion

		Bacter	ia only	Bacteria and alga			
Days	Temper- ature ° C.	Bacteria in 1 cc (in thousands)	D. O., p. p. m.	Bacteria in 1 cc (in thousands)	D. O., p. p. m.	Alga in 1 cc (in p. p. m. by vol- ume)	
(1)	(2)	(3)	(4)	(5)	(6)	m	
0	20. 5 20. 5 21. 5 19. 5 20. 5 19. 5 20	8. 6 2, 270 7, 900 7, 500 8, 200 8, 350 7, 350	8. 45 6. 50 5. 96 5. 38 5. 22 5. 18 5. 50	8. 5 272 8, 300 8, 700 7, 600 9, 300 9, 050	8. 30 7. 98 5. 72 5. 36 5. 50 5. 38 6. 32	2. 56 4. 31 3. 20 3. 52 4. 47 5. 60 8. 95	

¹ No sample.

Table 5.—History of dissolved oxygen in bacterial cultures with and without alga, with no atmospheric aeration meantime. In darkness, and without motion, in the 20° C. incubator

		Bacter	ia only	Bacteria and alga			
Days	Temper- ature ° C.	Bacteria in 1 cc (in thousands)	D. O., p. p. m.	Bacteria in 1 cc (in thousands)	D. O., p. p. m.	Alga in 1 cc (in p. p. m by vol- ume)	
(1)	(2)	(3)	(4)	(5)	(6)	(7)	
0	20. 5 20 21 21 21. 5 20. 5 20 22. 5	8. 6 770 8, 300 9, 450 8, 950 8, 150 8, 350 8, 100 8, 350	8. 45 7. 70 5. 87 6. 52 5. 50 5. 54 5. 62 5. 37 5. 38	8. 5 620 8. 750 8. 800 9. 100 9. 400 8. 200 7. 600 7, 000	8. 30 7. 80 5. 64 5. 58 5. 40 5. 42 5. 40 5. 40 5. 20	2. 56 3. 52 4. 16 3. 52 2. 40 3. 20 3. 68 2. 72 2. 40	

The general set-up of experiment no. 2 (as to organisms, medium, and containers) was the same as in experiment no. 1. The procedure followed was also the same, but an added feature was the placing of a set of cultures in the 20° C. incubator. (See table 5.)

In the bacteria-only culture, in darkness and without motion, in the 20° C. incubator (see table 5), essentially the same course of events takes place as in the preceding bacteria-only cultures, table 3, in the light. Bacterial maximum is reached the third day, after which a very slight decrease takes place, but bacterial content remains at a relatively high point until the close. The dissolved oxygen content is similarly stabilized after the first drop (in 48 hours) from the initial content caused by the bacteria increasing to maximum.

The bacteria-plus-alga portion of table 5 is worthy of study. The bacterial history of these cultures stored in darkness in the 20° C. incubator is in all respects a practical repetition of that in the com-

panion cultures in sunlight and containing bacteria only. The dissolved oxygen history of the two set-ups in table 5 (bacteria only, and bacteria-plus-alga) is likewise an item for item proposition. The algal history meantime, after a temporary and very slight increase over the initial content of 2.56 p. p. m., shows a somewhat erratic course to a final value of 2.40 p. p. m., which is slightly less than the initial content of 11 days previous. This is radically different from the algal histories of any of the four preceding set-ups, in all of which a progressive increase and heavy final algal content is to be found.

Summary.—1. There is no essential difference in algal growth or in dissolved oxygen history in the recorded results in tables 3, 4, and 5 to indicate any marked advantage resulting from continuous mixing.

- 2. Decrease in dissolved oxygen is correlated, in time and substantially in magnitude, with the increase in bacterial content in all three of the bacteria-only experiments, regardless of motion and of light.
- 3. In the companion cultures (containing both bacteria and alga) of the two set-ups exposed to light, tables 3 and 4, the dissolved oxygen shows a drop from the initial content at the time of greatest bacterial increase, practically paralleling, in time and magnitude, the similar event in the bacteria-only cultures. These bacteria-and-alga cultures then show a slow, but well-marked, *increase* of dissolved oxygen, failing, however, to regain the initial content. In the similar culture exposed to darkness (table 5) the drop of dissolved oxygen from the initial content is indicated, but the later increase and attempted recovery is absent.
- 4. Growth of alga in the two set-ups exposed to light shows a progressive increase over the initial content. In the similar culture exposed to darkness, a very slight and temporary increase is noted (see table 5), with subsequent decrease to a point slightly less than the initial content.
- 5. Algal cells in cultures stored in darkness showed scant growth and failed to produce oxygen. The same alga during 10 percent of sunshine hours in experiment no. 2 showed moderate increase (400 percent) and produced nearly enough oxygen to replace that consumed by bacterial growth; but in experiment no.1, with 19 percent sunshine hours, algal growth was heavy (4,000 to 5,000 percent), and the oxygen thus produced was far more than enough to replace the amount consumed.
- 6. There is noticeable smoothness in the curves of increase or of decrease indicated by the data in table 5, in bacteria and in dissolved oxygen (see columns 3, 4, 5, and 6), a logical result of stable temperatures and absence of sunlight. The values in the alga content (column 7), are erratic in comparison.

Experiment no. 3.—February 24 to March 6, 1931, inclusive.

During the 10 days of the experiment there were 10 half-days (40 hours) of sunshine and 10 half-days (40 hours) of cloudy and smoky daylight, during which latter period photosynthesis must necessarily have been greatly limited. The 40 hours of effective light (sunshine) constituted 18½ percent of the total 240 hours' duration of the experiment.

The medium in experiment no. 2 (preceding) was slightly cloudy, owing to precipitation at the time of autoclaving. The medium in experiment no. 3 was clear. The pH determined at start, midway, and at the close of the 10-day experiment was 7.3 for all three of the bacteria-only set-ups, and 7.1 for all three of the companion set-ups, which contained both bacteria and alga.

Results of experiment no. 3 are recorded in tables 6, 7, and 8.

All three of the bacteria-only cultures followed the same general course as that in the preceding two experiments as to bacterial content and dissolved oxygen consumption. There was no well-marked replacement of oxygen.

Table 6.—History of dissolved oxygen in bacterial cultures with and without alga, with no atmospheric aeration meantime, in sunlight, and without motion

		Bacter	ia only	Bacteria and alga			
Days	Temper- ature °C.	Bacteria in 1 cc (in thousands)	D. O., p. p. m.	Bacteria in 1 cc (in thousands)	D. O., p. p. m.	Alga in 1 cc (in p. p. m. by vol- ume)	
. (1)	(2)	(3)	(4)	(5)	(6).	(7)	
0	20. 5 26 26 25 22. 5 18 19 18. 5	16. 6 4, 506 6, 100 7, 300 4, 800 6, 000 700 510	8. 86 6. 84 6. 18 6. 30 6. 84 7. 04 7. 00- 7. 72	16. 6 4, 100 8, 200 6, 900 160 7, 000 2, 240 940	8. 92 6. 70 7. 04 8. 78 9. 92 11. 88 12. 80 11. 44	2. 24 2. 62 4. 10 11. 70 39. 20 49. 40 72. 00 72. 60	

Table 7.—History of dissolved oxygen in bacterial cultures with and without alga, with no atmospheric aeration meantime, in sunlight, and with continuous motion

		Bacter	ia only	Bacteria and alga			
Days	Temper- ature °C.	Bacteria in 1 cc (in thousands)	D. O., p. p. m.	Bacteria in 1 cc (in thousands)	D. O., p. p. m.	Alga in 1 cc (in p. p. m. by vol- ume)	
(1)	(2)	(3)	(4)	(5)	(6)	(7)	
0	20. 5 26 26. 5 26 23 19. 5 21 20. 5	16. 6 5, 040 7, 550 7, 300 8, 600 3, 730 4, 800 2, 890	8. 86 6. 30 5. 94 5. 64 6. 08 5. 50 6. 18 6. 30	16 4, 910 7, 150 9, 100 6, 100 5, 200 1, 980 1, 090	8. 92 6. 52 7. 27 7. 36 8. 46 9. 78 12. 20 12. 08	2. 24 2. 84 4. 7 10. 3 24 35 75. 7 67. 7	

Table 8.—History of dissolved oxygen in bacterial cultures with and without alga, with no atmospheric aeration meantime, in darkness, and without motion, in the 20° C. incubator

		Bacter	ia o nly	Bacteria and alga			
Days	Temper- ature °C.	Bacteria in 1 cc (in thousands)	D. O., p. p. m.	Bacteria in 1 cc (in thousands)	D. O., p. p. m.	Alga in 1 co (in p. p. m. by vol- ume)	
(1)	(2)	(3)	(4)	(5)	(6)	(7)	
0	20. 5 21. 5 22. 5 22. 5 23 20 22 21. 5	16. 6 4, 360 7, 000 8, 800 9, 150 7, 450 8, 900 6, 800	8. 86 6. 74 6. 38 6. 28 6. 80 5. 96 6. 14 6. 08	16 4, 800 9, 400 8, 550 9, 400 7, 900 8, 600 7, 500	8. 92 6. 62 6. 40 6. 32 6. 28 6. 10 6. 68 5. 94	2. 24 2. 75 2. 55 2. 24 2. 68 2. 14 2. 14 1. 66	

The two cultures containing both bacteria and alga cells, and exposed to light meantime, show bacterial content very similar to that in the bacteria-only cultures, but the dissolved oxygen history (column 6, tables 6 and 7) shows a marked replacement of oxygen, the final amount greatly exceeding the initial content in both cases. Meantime the alga cells have increased by about 3,000 percent. The bacteria-and-alga cultures in darkness show the usual bacterial history (see table 8). The dissolved oxygen history shows the usual drop from the initial content, but does not recover. It is apparent that the alga cells have not functioned, for there is neither increase of these cells nor replacement of dissolved oxygen.

Experiment no. 4.—In this experiment the culture bottles were provided with the individual seal of sterile water held about the stopper and neck of the bottle by a superimposed finger cot, as previously explained. We also added a third group of bottles containing the same medium as the others and subject to the same technique in all respects, but inoculated with alga cells only.

During the period April 6 to 23, inclusive, there were 25½ half-days of sunshine and 8½ half-days of cloudy weather. Regarding 8 a. m. as the hour at which sunlight is sufficiently effective for plant activity, and 4 p. m. as the approximate time of the end of such activity, the 17 days' duration may be stated as a total of 408 hours, of which 25½ half-days of sunshine (of 4 hours each) give a total of 102 hours. Thus, the approximate amount of effective sunlight comprised 25 percent of the total 408 hours.

The results of experiment no. 4 are recorded in tables 9, 10, and 11.

TABLE 9.—History of dissolved oxygen in cultures of bacteria only, of bacteria and alga, and of alga only, with no atmospheric aeration meantime. In sunlight, and without motion

		Bacteri	a only	Bac	teria and	alga	Alg	a only	
Days	Tem- pera- ture, °C.	Bacteria in 1 cc (in thou- sands)	D. O., p. p. m.	Bacteria in 1 cc (in thou- sands)	D. O., p. p. m.	Alga in 1 cc (in p. p. m. by vol- ume)	D. O., p. p. m.	Alga in 1 cc (in p. p. m. by vol- ume)	Remarks
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
0	22 22. 5 22. 5 30 23 26 27. 5 29 22. 5 30. 5	18. 9 6. 030 10, 800 11, 000 11, 100 12, 000 8, 550 4, 460 6, 700 1, 620	8. 52 4. 48 3. 48 3. 22 2. 22 2. 52 1. 82 0. 42 (1)	22. 6 6, 140 10, 400 11, 000 11, 400 14, 000 6, 550 3, 370 5, 300 1, 390	8.50 4.96 4.22 5.08 4.50 4.80 4.48 5.12 4.63 6.34	4. 4 10. 3 10. 4 24. 2 30. 6 44. 5 44. 6 56. 9 19. 7 26. 6	8. 40 8. 76 • 7. 70 • 6. 94 12. 76 • 13. 20 • 13. 20 12. 90 13. 36	5. 4 14. 1 • 17. 2 • 48. 0 87. 0 90. 8 • 137. 6 125. 4 111. 4	• Contaminated.

¹ Depleted.

Table 10.—History of dissolved oxygen in cultures of bacteria only, of bacteria and alga, and of alga only, with no atmospheric aeration meantime. In sunlight, and with continuous motion

		Bacteri	a only	Bac	teria and	alga	Alg	a only	
Days	Tem- pera- ture, °C.	Bacteria in 1 cc (in thou- sands)	D. O., p. p. m.	Bacteria in 1 cc (in thou- sands)	D. O., p. p. m.	Alga in 1 cc (in p. p. m. by vol- ume)	D. O., p. p. m.	Alga in 1 cc (in p. p. m. by vol- ume)	Remarks
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
0	22 27 25 28. 5 24 24 26 26. 5 23. 5 29	18. 9 8, 010 12, 500 10, 900 10, 600 15, 400 11, 200 9, 600 6, 100 5, 200	8. 52 4. 18 2. 90 2. 84 2. 50 2. 26 1. 98 1. 36 0. 04 0. 38	22. 6 6, 370 12, 500 11, 600 11, 000 12, 060 11, 000 9, 500 9, 100 2, 220 2, 630	8.50 4.38 4.00 4.48 1 2.18 5.04 5.10 5.58 5.92 8.00 7.54	4. 4 12. 2 12. 1 23. 8 17. 7 19. 6 34. 5 34. 5 24. 7 27. 3 26. 0	8. 40 8. 52 9. 02 12. 38 11. 58 12. 60 12. 66 12. 18 13. 03 12. 18 14. 56	5. 4 9. 1 16. 5 61. 2 99. 3 96. 6 145. 2 143. 5 112. 5 131. 2	• Light bacterial contamination at time of examina- tion.

¹ There is no apparent explanation for this low value in D. O.

Table 11.—History of dissolved oxygen in cultures of bacteria only, of bacteria and alga, and of alga only, with no atmospheric aeration meantime. In darkness, and without motion, in the 20° C. incubator

		Bacteri	a only	Bac	teria and	alga	Alg	a only			
Days	Tem- pera- ture, °C.	Bacteria in 1 cc (in thou- sands)	D. O., p. p. m.	Bacteria in 1 cc (in thou- sands)	D. O., p. p. m.	+Alga in 1 cc (in p. p. m. by vol- ume)	D. O., p. p. m.	Alga in 1 cc (in p. p. m. by vol- ume)	Remarks		
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)		
0	22 22, 5	18.9 7, 230	8. 52 4. 32	22. 6	8. 50	4. 4	8. 40	5. 4	Available samples were too few to		
2	22	13, 000	3.48						permit examina-		
3	24	10, 400	3. 18						tion each day,		
4	23.5	12, 500	3. 16	11,360	3.08	9. 2	7.38	26.5	hence initial, 4th,		
5	23	12, 000	3.10						11th, and 17th		
7	19	15, 000	2.90						days'. Samples		
9	22	11, 100	2.72						were selected as		
11	22	11, 800	2.70	9, 750	2,60	15.7	• 1.88	4 33. 1	representative.		
14	24	9,000	2.58						 Contaminated 		
17	21.5	8, 050	2.38	8, 850	2.04	8.5	4.98	41.4	with bacteria.		

² Depleted.

In the bacteria-only cultures in darkness (table 11) bacterial growth is of about the same magnitude as in the similar cultures exposed to light, but the amount of oxygen used meantime is somewhat less—only 6.14 parts—and the initial content of 8.52 parts is, therefore, sufficient to save these cultures from the fate of oxygen depletion experienced by the similar cultures exposed to light. This difference in amount of oxygen consumed may be due in part to the fairly stable temperatures (column 2) in the incubator as compared with the widely varying day temperatures of light-exposed cultures in tables 9 and 10.

Summary.—1. Cultures containing bacteria only are found to deplete, wholly or in large part, the initial content of dissolved oxygen.

- 2. Cultures containing both bacteria and alga show similar heavy decrease of initial dissolved oxygen, but in the presence of sunlight later regain nearly all of it because of the output of plant-made oxygen by the increase of 2,000 percent in algal content.
- 3. Bacteria-only cultures in darkness (in the 20° C. incubator) show a bacterial content similar in day-by-day magnitude to the cultures exposed to light, but the dissolved oxygen is not exhausted, though heavily reduced.
- 4. Bacteria-and-alga cultures in darkness show a bacterial history very similar in all respects, and the dissolved oxygen is similarly reduced, but without any tendency toward final recovery, the alga meantime increasing only a very little. The alga-only cultures show an essentially similar oxygen history.

Experiment no. 5.—The objects were as follows:

- 1. To repeat the work done in experiments 1, 2, 3, and 4, and thus to obtain additional data relative to the ability of a unicellular alga in pure culture to provide, by photosynthesis, sufficient dissolved oxygen to meet the requirements of aerobic bacteria increasing to moderately high numbers in a medium simulating a diluted sewage, atmospheric aeration being cut off meantime.
- 2. Using as a background the accumulated roughly quantitative data as to the positive performance of the alga in providing oxygen without recourse to atmospheric aeration, to present, for comparison, the bacterial history, the alga history, and especially the dissolved oxygen history of exactly similar cultures, run at the same time, but differing in the one particular that these similar cultures were exposed to continuous atmospheric aeration, but under aseptic conditions. The bottles in this portion of Experiment 5 were placed in the arrangement of balanced movable hanging shelves already described as the "elevator." Other cultures were placed, as usual, in the rotating cage, where they had both motion and sunlight, and on the stationary shelf, where they had sunlight but no motion. No cultures were run in the 20° incubator in this experiment.

During July 12-26, 1932, there were 24 sunny half days and 4 cloudy (light overcast) half-days, or 120 hours of effective light and 20 hours of noneffective (or less effective) light, owing to partial overcast of clouds. This is on the basis of the hours 7 a. m. to 5 p. m. being regarded as effective light. In terms of percent, 86 percent of the daylight was effective as compared with 14 percent noneffective. In terms of the total of 336 hours of this 14-day experiment, effective sunlight made up almost 36 percent of this total. No definite record was kept of local smoke clouds, or of winds which, if present, quickly dispersed such clouds.

Tables 12, 13, and 14 record the results, the first two giving the data of the sealed cultures, with atmospheric aeration excluded, and table 14 giving results from exactly similar cultures which were exposed, during the entire time of the experiment, to atmospheric aeration and to mixing of bottle contents.

Table 12.—History of dissolved oxygen in cultures of bacteria only, of bacteria and alga, and of alga only, with no atmospheric aeration meantime. In sunlight and without motion

		Bacteri	a only	Bac	teria and	alga	Alg	a only		
Days	Tem- pera- ture, °C.	Bacteria in 1 cc (in thou- sands)	D. O., p. p. m.	Bacteria in 1 cc (in thou- sands)	D. O., p. p. m.	Alga in 1 cc (in p. p. m. by vol- ume)	D. O., p. p. m.	Alga in 1 cc (in p. p. m. by vol- ume)	Remarks	
(1)	(2)	(3)	(4)	(5) (6)		(7)	(8)	(9)	(10)	
0	22. 5 30 34 36 36 35. 5 36 35. 5	34. 3 6, 600 9, 800 8, 500 6, 300 364 28. 6	7. 1 3. 6 2. 5 2. 0 0. 9 0. 9 0. 7 1. 1	34. 6 5, 500 9, 050 6, 500 750 375 20 73. 5	7. 0 3. 6 2. 58 3. 18 2. 32 3. 22 5. 12 7. 08 6. 90	4. 2 7. 3 10 12. 7 10. 2 13	7. 1 7. 02 8. 40 9. 00 10. 90 6. 82 10. 56 2. 4 10. 22	2. 9 8. 2 16. 5 17. 4 30. 5 16. 7 36. 1 20. 2	Cause of low D. O. unknown Contaminated.	

Table 13.—History of dissolved oxygen in cultures of bacteria only, of bacteria and alga, and of alga only, with no atmospheric aeration meantime. In sunlight, and with continuous motion

		Bacteri	a only	Bac	teria and	alga	Alg	a only	: : ;
Days	Tem- pera- ture, °C.	Bacteria in 1 cc (in thou- sands)	D. O., p. p. m.	Bacteria in 1 cc (in thou- sands)	D. O., p. p. m.	Alga in 1 cc (in p. p. m. by vol- ume)	D. O., p. p. m.	Alga in 1 cc (in p. p. m. by vol- ume)	Remarks
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
0 1 2 3 4 6 8 11 14	22. 5 30 37 36 35 34 33. 5 28	34. 3 5, 650 9, 600 7, 500 3, 750 1, 220 151 162 28. 8	7. 1 3. 6 2. 4 1. 1 0. 72 0. 30 (1) (2) (1)	34. 6 6, 200 9, 600 5, 850 1, 020 405 350 152 40	7. 0 3. 8 2. 8 2. 42 2. 8 4. 28 5. 82 8. 62 7. 58	4. 2 9. 4 9. 1 8. 8 8. 2 16. 5 21. 7 16. 2 18. 5	7. 1 • 7. 1 7. 8 • 4. 0 • 4. 2 • 5. 92 10. 0 11. 2 11. 94	2.9 • 7.3 13.1 • 7.9 • 13 • 16.9 37.8 39.5 14.7	• Contaminated.

Table 14.—History of dissolved oxygen in cultures of bacteria only, of bacteria and alga, and of alga only, with atmospheric aeration meantime. In sunlight, with motion, every 6 minutes, in the elevator

		Bacteri	a only	Bac	teria and	alga	Alg	a only		
Days	Tem- pera- ture, °C.	Bacteria in 1 cc (in thou- sands)	D. O., p. p. m.	Bacteria in 1 cc (in thou- sands)	D. O., p. p. m.	Alga in 1 cc (in p. p. m. by vol- ume)	D. O., p. p. m.		Remarks	
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	
02 23 46 811	22. 5 31 36 36 38 32 35 33 28. 5	34. 3 5, 950 10, 600 9, 150 1, 260 1, 010 198 14. 1 67	7. 1 5. 35 5. 78 5. 98 5. 88 5. 92 6. 62 6. 50 6. 90	34. 6 6, 200 10, 000 5, 750 940 645 248 198 117	7. 0 5. 20 5. 76 6. 00 6. 52 7. 04 6. 98 6. 80 7. 40	4. 2 6. 8 15. 7 10. 3 9. 3 16. 5 17. 8 16. 1 19. 1	7. 1 7. 15 5. 74 8. 32 7. 38 6. 92 7. 00 6. 82 7. 40	2. 9 7. 7 • 24 36 28. 3 34. 6 35. 2 39. 4 43. 5	• Contaminated.	

Table 14 presents the results obtained from cultures in the elevator, which cultures, in paired bottles, stoppered with cotton plugs, were exposed to atmospheric aeration through a thick mat of sterilized cotton. The contained liquid was mixed through an overhead siphon once in 6 minutes, as previously explained. In all other respects these cultures were exactly like those in the rotating cage, or those without motion on the shelf.

It will be noted in column 9, table 14, that the increase of alga cells is considerable, from an initial value of 2.9 parts to a final of 43.5 parts, yet the dissolved oxygen values are not appreciably larger than those in column 6, the cultures of which contain (see column 7) fewer alga cells. Looking into this matter a little further we may compare the results of alga growth in tables 12 and 14, respectively, excluding any contaminated cultures, as follows:

Taking the alga-only cultures of each, we note that in columns 8 and 9, in table 12, 7 cultures show an average content of 26.9 parts per million (by volume) of alga cells. These same cultures show a total accumulation, in 14 days, of 3.12 parts per million of alga-made oxygen (the initial content having been deducted). Meantime, as shown in table 14, the air-exposed cultures, similarly considered, indicate an average content of 32.1 parts per million of alga cells. These cells apparently produce, in 14 days, an accumulation of oxygen amounting to only 0.30 parts per million, or about one-tenth as much as was produced in the sealed cultures.

Apparently the only reasonable explanation of this discrepancy is the assumption that, in these unsealed cultures, some of the plantmade oxygen escaped to the air.²

³ Birge and Juday, studying Lake Mendota, found excess plant-made oxygen only during calm weather. A breeze, setting the surface water in circulation, caused escape of excess oxygen to the air. (See p. 51 of reference no. 5).

Summary.—In brief summary of this final experiment, we submit the following items:

- (1) Heavy decrease of the initial dissolved oxygen occurs in those sealed cultures which contain bacteria only.
- (2) Sealed cultures containing both bacteria and alga cells record a similar drop in the initial dissolved oxygen at the time of greatest bacterial increase, but the growth of alga cells meantime produces sufficient oxygen to replace practically all of the consumed oxygen.
- (3) Sealed cultures containing alga cells only show no decrease in the initial dissolved oxygen content, but a fairly uniform increase instead, obviously due to the output of oxygen from the alga.
- (4) Cultures unsealed, but stoppered with cotton plugs, thus providing contact with the atmosphere, show, in bacteria-only cultures, a drop from the initial dissolved oxygen only half as great as the decrease shown by the sealed cultures meantime, though both cultures have similar bacterial content. Instead of further gradual depletion, as in the sealed cultures, these air-exposed cultures maintain a moderate oxygen content throughout the remaining days of the experiment.
- (5) Similar unsealed and air-exposed cultures which contain both bacteria and alga cells show a dissolved oxygen history which is practically identical in all respects to the oxygen history stated in item (4) of those cultures which contain no alga cells. The bacterial content meantime is of about the same magnitude in each.
- (6) Unsealed and air-exposed cultures containing no bacteria, but alga cells only, record no drop at all from the initial dissolved oxygen content. Neither is there any material increase in this content, notwithstanding a 1,400 percent increase of the contained alga cells. With but slight fluctuations, the initial content of dissolved oxygen is maintained throughout.
- (7) The greatly differing dissolved oxygen history of all these air-exposed cultures, as compared with the like history of similar but sealed cultures, is apparently due to contact with the atmosphere, this stabilizing body supplying oxygen to some cultures when a partial deficit exists, or receiving the excess plant-made oxygen given off by other cultures, thus maintaining the oxygen content of all at a fairly uniform level. Atmospheric oxygen thus functions as an equalizing reservoir.
- (8) Bacterial content is relatively low. It seems possible that the hot July sun, which developed a mean temperature of 33° C. within the cultures, is also the sufficient explanation of an intensity of sunlight which tended to kill some of the bacteria, as indicated in column 5 of tables 12 and 13.

DISCUSSION

The tabulated results of the foregoing experiments furnish reasonable evidence as to the replacement of dissolved oxygen used by bacterial growths, thus balancing and stabilizing the biochemical machine.

Tables 15 and 16 summarize the like data from 24 sets of cultures as recorded in 10 of the tables already given (nos. 1, 2, 3, 4, 6, 7, 9, 10, 12, and 13), and by averaging the daily results as there given, tables 15 and 16, recording those averages, are constructed (two tables are used because of differing dates of sampling after the fourth day). This final summary of the net results is further depicted by the two accompanying graphs.

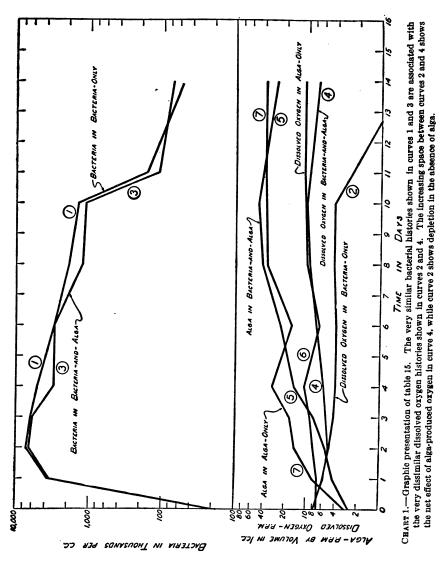
Table 15.—Summary of data from tables 1, 2, 6, 7, 12, and 13. Averages showing history of dissolved oxygen in 6 cultures of bacteria only, 6 cultures of bacteria and alga, and 2 cultures of alga only, with no atmospheric aeration meantime

	Bacter	ia only	Ba	cteria and	alga	Alga	only
Days	Bacteria in 1 cc (in thou- sands)	D. O., p. p. m.	Bacteria in 1 cc (in thou- sands)	D. O., p. p. m.	Alga in 1 cc (p. p. m. by volume)	D. O., p. p. m.	Alga in 1 cc (p. p. m. by volume)
(1)	(2)	(3)	(4)	(5)	(6)	m	(8)
0	19. 4 3, 709 7, 165 6, 265 5, 173	7. 89 5. 80 4. 87 4. 25 4. 08	19. 2 3, 572 6, 537 5, 915 3, 072	7. 91 5. 69 5. 04 5. 53 5. 92	2. 57 4. 33 5. 29 8. 10 15. 21	7. 10 7. 02 8. 10 9. 00 10. 90	2. 90 8. 20 14. 80 17. 40 30. 50
6 7	3, 042	4. 05	3, 022	7. 58	23. 73	6.82	16. 70
8 9	1, 924	4. 38	1, 252	9. 51	42. 48	10. 28	36. 95
10 11 14	4, 448 162 53	4. 32	1, 133 113 73	10. 65 7. 24	49. 33 27. 65	11. 20 11. 08	39. 50 38. 85

Table 16.—Summary of data from tables 3, 4, 9, and 10. Averages showing history of dissolved oxygen in 4 cultures of bacteria only, 4 cultures of bacteria and alga, and 2 cultures of alga only, with no atmospheric aeration meantime

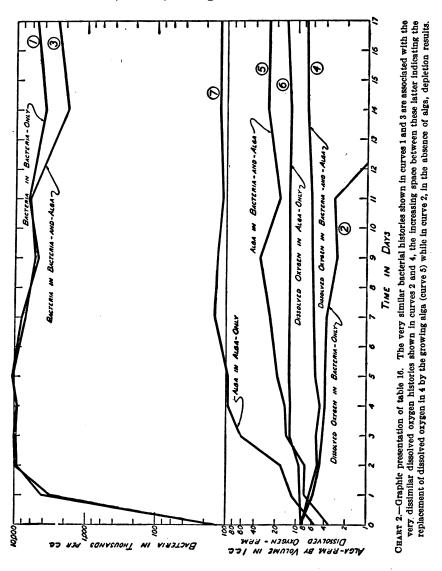
	Bacter	ia only	Ba	cteria and	alga	Alga	only
Days	Bacteria in 1 cc (in thou- sands)	D. O., p. p. m.	Bacteria in 1 cc (in thou- sands)	D. O., p. p. m.	Alga in 1 cc (p. p. m. by volume)	D. O., p. p. m.	Alga in 1 cc (p. p. m. by volume)
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
0	13. 8 4, 180 9, 275 9, 513 9, 125 10, 613	8. 49 5. 77 4. 68 4. 25 3. 93 3. 94	15. 6 3, 156 9, 813 10, 025 9, 338 10, 525	8. 40 6. 41 4. 92 5. 19 4. 60 5. 33	3. 48 7. 66 7. 51 13. 96 14. 56 18. 79	8. 40 8. 64 9. 02 12. 38 12. 17 12. 75	5. 40 11. 60 16. 50 61. 20 93. 15 93. 70
7 8	8, 025	3. 75	7, 070	5. 63	24. 01	12. 66	145. 20
9 10	4, 667	2. 69	5, 207	5. 93	33. 57	12.69	134. 45
11 14 17	6, 523 3, 860 5, 200	2. 94 0. 19 0. 00	6, 213 1, 805 2, 630	6. 19 7. 17 7. 54	17. 70 26. 95 26. 00	12. 96 12. 77 14. 56	111. 95 124. 25 126. 00

In our experiments, our chief object was to learn the trend of such results and the direction of such change as might be expected to occur during natural weather conditions and in the natural waters with which we ordinarily are concerned. The outstanding and persistent change noted (i. e., the measurable quantity of oxygen produced by a



relatively small volume of algal cells), is significant not only because of the great importance of this oxygen to the polluted water, but also because of the prevalence of conditions meantime in our experiments, which are mainly natural rather than artificial. This refers to (1) such sunlight as was available during these several periods of experi-

mentation, (2) such temperature as the natural weather provided, and (3) such dissolved organic content as is comparable to a heavily-polluted water in nature. Similarly (4), the content of algal cells was not greater than that which we commonly find in such sun-exposed waters in nature; in fact, the alga content of a natural water is often



much higher than that of our cultures. On the other hand, proper checking of our results necessitated certain limitations not found in nature. Chief of these were (1) limiting the amount of any one culture to 300 cubic centimeters, (2) enclosing this within a bottle and sealing to prevent contact with external air, (3) passing of sunlight

through window glass and also through the glass sides of the bottle, before reaching the organisms of the culture, and (4) limiting the contained organisms to one kind only, or to two kinds at most.

Our claim, therefore, is not for mathematical exactness, but rather for the inevitable direction and relative amount of change involving the dissolved oxygen content of such waters as contain even moderate quantities of algal cells and are exposed to such sunlight as is afforded by ordinary weather conditions. The quantitative note is persistent and unmistakable by the production, in every instance (except in those cultures incubated in darkness) of sufficient amounts of this algamade oxygen to replace nearly or quite all that has been consumed by the rising bacterial content, and thus the disaster of oxygen depletion is prevented.

Approximate unit production of oxygen by alga cells.—While the exact quantity of oxygen produced by photosynthesis is a complicated problem (23, 24) involving many factors, yet, we have, in the tables presented herewith, sufficient data to approximate the quantity of oxygen formed by a representative unicellular alga under conditions of temperature, available sunlight, and degree of pollution fairly typical of the average situation in nature. In partial answer to the question, What may we expect of the algae usually present in a natural water body? we submit the following items:

- A. Production in cultures of alga only;
- B. Production in cultures of alga-and-bacteria.

A. The alga-only sections of tables 9, 10, 12, and 13 furnish data by which we may approximate the quantitative production of oxygen by the alga used.

Utilizing for the present that portion of any given table which records uninterrupted day-to-day values, we note, in table 9:

The initial dissolved oxygen content of 8.40 parts increases in 5 days to 12.90 parts, indicating that the alga present has produced 4.50 parts.

The average content of alga in the meantime (omitting two contaminated samples) is 63.97 parts.

This average content of alga producing a known amount of oxygen in a known number of days gives all the data required to find that, in 1 day, 1 part per million of alga produces 0.014 parts per million of oxygen.

In like manner, we find the amount of oxygen produced per part per million of alga per day in tables 10, 12, and 13. The results are recorded in the final column of table 17.

Table 17.—Unit production of oxygen in the alga-only portions of tables 9, 10, 12, and 13

		Dis	solved oxy	Average alga con-	Oxygen pro- duced per	
Table	Consecu- tive days	Initial	Final	Produced by alga	tent (p. p.	day per p. p. m. of alga (p. p. m.)
9	5 5 4 2	8. 40 8. 40 7. 10 7. 10	12. 90 12. 60 10. 90 7. 80	4. 50 4. 20 3. 80 . 70	63. 97 56. 54 18. 15 10. 05	0. 014 . 015 . 052 . 035

B. Seeking similar information in the sections relating to bacteria and alga of tables 1, 2, 3, 4, 6, 7, 9, 10, 12, and 13, respectively, it becomes necessary to expand each table in order to make accessible the somewhat involved data. For example, table 9 becomes:

Bacteria only Bacteria and alga Dissolved oxy-Dissolved oxygen gen (p. p. m.) Day Bacteria Bacteria Alga in Temin 1 cc (in thouin 1 cc (in thou-1 cc (p. p. m. by pera-Total ture C From From Consands) Used sands) Used convolume) tent initial alga tent (B) (1) (2) (3) (4) (A) (5) (6) (C) (D) (7) 22 22. 5 22. 5 30 23 18.9 8. 52 22.6 6, 030 10, 800 11, 000 11, 100 4.04 1.00 .26 1.00 6, 140 10, 400 11, 000 4. 10 . 97 . 26 4. 48 3. 48 4. 96 4. 22 4. 40 8. 43 10. 3 3. 22 2. 22 5.08 4.50 3. 17 1.91 1.03 2.36 2.31 11, 400 12,000 2. 52 -. 30 14,000 4.80 2 49 2. 53 27.5 8,550 1.82 . 70 6,550 . 54 4.48 1.95 29 4,400 . 42 1. 40 3, 370 1.07 5. 12 . 88 4. 24 6, 700 . 42 5. 300 4. 63 . 55 4.08 22. 5 Depl. . 33 24. 3 30.5 1,620 Depl. . 0 1, 390 . 0 6. 34 . 55 5. 79

Table 18.—Expansion of Table 9

The "expansion" consists in introducing columns A, B, C, and D. (The numbered columns 1 to 7, inclusive, are identical with those in the original table 9.)

Column A is derived by tabulating day by day the amount of oxygen used, as shown by the daily decrease in column 4.

Column B is derived by ascertaining the proportional amount of oxygen used by the daily bacterial content shown in column 5, as compared with the known amounts (see column A) used by the similar daily bacterial content in column 3. By using for each day's data, the proportion column 3: column 5=column A: column B, the amount for each day in column B is found. For example, 6030:6140=4.04:x (=4.10).

¹ Extrapolated value.

Since the total amount of dissolved oxygen present (column 6) consists in part (1) of that present at the start (initial) and in part (2) of oxygen made by the alga during the days of the experiment, these two components may be differentiated by recording, in column C, the successive amounts remaining after deducting, from the initial content, the first-day item in column B, then from the remainder deducting the next successive item in column B, and so on. The resulting column C shows each day's remnant of such oxygen as was present at the start (initial content).

But the total amount of oxygen found to be actually present each day (column 6) is usually more than that shown in the items in column C. The alga is the only possible source of this additional oxygen; and as the alga increases from day to day (column 7), the amount of algamade oxygen should increase also. By subtracting each item in column C from the item of the same date in column 6, the daily record of these differences is obtained, and this is recorded in column D. These same items represent the successive daily accumulations of alga-made oxygen, since, in the sealed bottles, there was neither access of atmospheric oxygen nor escape of such oxygen as was generated by alga within the bottles. The oxygen present in column D on any given day (for example, day 4) represents not only the oxygen formed on that day, but also the accumulated oxygen formed on preceding days.

The alga, introduced into cultures at the start in very small amount, increases much more slowly (see column 7 in tables 1, 2, 3, 4, 6, 7, and 13) than do bacteria, with the result that only very small amounts of alga-made oxygen are present for 3 or 4 days. These small amounts may even be within the range of experimental error, and in several instances negative values are indicated. Under such conditions only approximate values are warranted, and the trend of the experiment as a whole is probably the only data of actual value (see table 19). An attempt to deduce reliable values from the very small and frequently negative day-to-day quantities furnished by the small but increasing quantities of alga in our experiments would seem unwarranted. Temperature fluctuations in particular are much in evidence and constitute a hazard in the attempt to interpret dissolved oxygen values when expressed in terms of parts per million.

The day-to-day results recorded in table 20 are submitted, but with realization of the doubtful value of the data, as previously stated.

The experiments as carried out result in incomplete tables in that certain days are omitted in order to conserve the limited number of cultures and prolong the experiment thereby. This incompleteness is a feature of tables 1 to 14, inclusive.

By recourse to extrapolation, the missing values have been supplied (see note to table 18) in order to arrive at a better established value to represent the daily production of oxygen by a given quantity of alga.

The extrapolation has been applied, in each expanded table, only to such data (column 7 in table 18) as was necessary in order to compute the desired value. Knowing (1) the average alga content per day, (2) the total amount of alga-made oxygen accumulated, and (3) the number of days required for this accumulation, we have accumulation of oxygen divided by average alga content, and the resulting quotient further divided by number of days of activity, giving the average quantity of oxygen produced per unit of alga per day. The results thus obtained are recorded in table 19.

Table 19.—Unit production of dissolved oxygen in the bacteria-and-alga section of tables 1, 2, 3, 4, 6, 7, 9, 10, 12, and 13

Experiment no.	Table	Approximate percent of sunlight	Average oxygen pro- duced per day per p. p. m. (by volume) of alga (Oöcys- tis) (p. p. m.)	Experiment no.	Table	Approximate percent of sunlight	Average oxygen pro- duced per day per p. p. m.(by volume) of alga(Oöcys- tis) (p. p. m.)
2	{ 1 2 3 4 4 6 7	19 19 10 10 18½ 18½	0. 043 . 028 . 027 005 . 01 . 02	5	{ 9 10 12 13	25 25 36 36	0. 014 . 017 . 02 . 04

Table 20.—Approximate production of oxygen per day by one part per million (by volume) of occystis cells. Daily results from cultures containing bacteria and alga

Day	Table 1	Table 2	Table 3	Table 4	Table 6	Table 7	Table 9	Table 10	Table 12	Table 13
1	-1.8 .6 .25 .14 .04	0 82 .03 .24 .20	0. 02 13 . 22 . 63 02 . 04	-0.03 45 .09 .07 04	-0. 16 . 27 . 21 . 04 . 02 . 01	0. 04 . 29 . 06 . 05 . 04 . 02	0. 08 . 02 . 06 . 02 001 . 003 	-0. 08 . 07 . 03 02 . 16 01 . 02	-0.08 09 06 04 08	0. 69 .03 .07 .06 .07 .06
17								003		

While the five experiments yield somewhat discordant data, it nevertheless seems fair to attach some importance to the tentative figures in tables 17 and 19, indicating the actual amount of reoxygenation from a given alga content working under ordinary day-to-day fluctuations of temperature and sunlight during natural weather. In every case the moderate content of algal cells sufficed to produce enough oxygen to replace practically all the oxygen used by the bacteria, in some cases even producing supersaturation, and in all cases avoiding, by a wide margin, the disaster of oxygen depletion.

Cultures in darkness (tables 5, 8, and 11) give significant results. In the absence of light, the algal cells apparently do not function, for the dissolved oxygen history in cultures which contain alga, together with bacteria (see column 6) is almost identical with that in the bacteria-only cultures (see column 4) in every one of these three experiments. In the one case (table 11, columns 8 and 9) where alga cells multiplied even in darkness, apparently this produced no oxygen, for the initial content of 8.4 parts was not increased, but was reduced, in 17 days, to about 5.0 parts, probably by respiration of the algal cells themselves.

Practical bearings.—Since the content of dissolved oxygen is a widely used measure in water examination, the possible source of such oxygen should be recognized and evaluated. Such evaluation must necessarily be guided by consideration of the relative abundance of submerged plants present and by the prevailing weather conditions as to sunlight. Diatoms sufficiently abundant to clog the filters may meantime be a valuable source of oxygen to that particular Night conditions of a polluted stream might be intolerable without the excess of oxygen previously provided by algae. Seasonal conditions are to a considerable extent secondary, because numerous algae and chlorophyll-bearing organisms are frequently abundant during cold weather; and even under a seal of ice, if this be clear enough to admit sunlight, certain organisms are apparently capable of producing sufficient oxygen to saturate the water, or to produce supersaturation if the ice seal prevents escape of excess oxygen to the air.

Marsh (25) found Synedra abundant in Lake Winnebago in the depth of winter for three successive winters. Knauthe (26), studying ponds covered with ice, records a dissolved oxygen content 515 percent saturation, this water containing in the meantime a very large growth of chlorophyll-bearing organisms. Olson (13), studying Minnesota lakes, records 115 percent saturation in the water of a lake sealed with clear, snow-free ice nearly 3 feet thick, through which green water plants were visible.

Probable relative absorption of plant-made oxygen and of atmospheric oxygen.—If algae be present, a measurable amount of pure oxygen is usually produced thereby within the water. Under natural conditions this water is in contact with the atmosphere, which is a mixture of gases, essentially 4 parts nitrogen and 1 part oxygen. The water, having a given "demand" for oxygen, will necessarily satisfy this demand by absorption from any stock or supply of oxygen available.

If this absorption takes place from the atmospheric supply at the water surface, such absorption must be in conformity with the fact that this oxygen, constituting about 21 percent of the atmosphere, is subject to a vapor pressure of only one-fifth that of pure oxygen. If absorption takes place from the alga-produced supply already within

the water, this supply, being pure, has a vapor pressure of about five times that of the atmospheric supply.

Discussing the amount of certain gases dissolved in natural waters, Whipple and Parker (22) say: "In a mixture of gases, the quantity of any one dissolved depends on the vapor pressure of that gas, regardless of the others. Thus a liter of water at 0° C. will dissolve 41.14 cc of oxygen if exposed to pure oxygen (at one atmosphere), but exposed to air (which is one-fifth oxygen) it will absorb 41.14 cc of oxygen under one-fifth the pressure, hence one-fifth as much by weight as in the first case."

In their exhaustive study of Wisconsin lakes, Birge and Juday (5) state (p. 25): "In a mixture of gases, such as the air, the absorption of each gas is independent of all the other gases present and is proportional to the pressure exerted by that gas."

Birge and Juday very frequently found the largest quantities of dissolved oxygen (100 to 300 percent saturation) not at the surface, but at depths varying from 1½ to 5 meters, at which depths, in these clear waters, there was sufficient light to enable the algae to function. In several of these instances the quantity of algae was considerably greater at these depths than in the surface waters.

Collection of the dissolved oxygen sample.—We would again point out, as we did some years ago (7), the very marked effect of submerged green plants, plus sunshine, on the dissolved oxygen content of the water and the resulting great difference in samples collected on sunny days as compared with cloudy-day samples, or afternoon samples as compared with morning samples. The time of collection must be selected with the same extreme care that we use in the selection of the place where the sample is to be collected. This essential fact has been further indicated in no uncertain terms by the studies of Rudolfs and Heukelekian (9), by Mohlman and his associates (8), by Streeter (20), by Calvert (14), by Olson (13), and by Hubbs (15). Not only should the deposits of decomposable sludge be recognized for their potential and actual effect on the oxygen expenditure of the stream, as contended by Mohlman and by Streeter, but the somewhat analogous content of resident submerged plant life and of microscopic algae should be adequately evaluated also, for their potential and actual effect on the oxygen income of the stream or water body concerned.

SUMMARY

- 1. Minute cells of green algae, sufficiently numerous to tint the water a scarcely perceptible green, and under average daily conditions as to sunlight, produced measurable amounts of dissolved oxygen.
- 2. The medium used simulated heavily polluted water, in that it contained sufficient dissolved organic matter to sustain a bacterial content which, in various separate experiments, reached maxima

varying from 5 millions to 13 millions per cc. However, only one kind of bacterium was present, and there were no protozoa, predatory or otherwise.

- 3. All cultures (those in table 14 excepted) were in completely filled bottles. Atmospheric aeration was eliminated. Duration of the experiments varied from 10 to 17 days.
- 4. Cultures containing bacteria but no alga cells showed serious decreases in dissolved oxygen, this sometimes being entirely depleted.
- 5. Cultures containing the minute alga in addition to the bacteria showed similar decrease of initial dissolved oxygen, but a program of recovery and replacement soon developed, becoming noticeable about the third day, when dissolved oxygen began to increase, coincident with increase in number of alga cells. This oxygen always prevented depletion by a wide margin, and sometimes produced supersaturation.
- 6. Cultures containing no bacteria, but alga cells only, showed no decrease of initial dissolved oxygen, but a progressive increase, corresponding in general with the daily increase in alga cells.
- 7. Check cultures in darkness in the 20° C. incubator showed (a) growth of bacteria similar to that in the cultures exposed to light, (b) no increase, or slight increase, of alga cells, (c) no replacement of dissolved oxygen.
- 8. Cultures having contact with the air show results very different from those shown by sealed cultures. The drop in initial oxygen is about one-third as great, then remains relatively stable, neither becoming depleted nor regaining an excess from algal activity. The alga-made oxygen as shown by titration is only about one-tenth the amount obtained from similar quantities of alga in the sealed cultures meantime. Apparently, exposure to the air, as obtains under conditions in nature, permits escape of most of the oxygen.

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MORTALITY AMONG SOUTHERN NEGROES SINCE 1920

Facts pertaining to the health of the Negro in the United States must be largely confined to statistics of mortality, since no large body of data on the incidence of illness among them is at present available. Mortality data, however, are published annually by the Bureau of the Census. In 1920 these reports embraced approximately twothirds of the Negro population, but the death registration area has been gradually extended, and in 1933 it included the entire country. The Public Health Service has recently issued a bulletin 1 summarizing

¹ Public Health Bulletin No. 235, prepared in the Office of Statistical Investigations by Dr. Mary Gover. This bulletin may be purchased from the Superintendent of Documents, Government Printing Office, Washington, D. C., at 10 cents per copy.

these data on Negro mortality. It is confined largely to Negroes of the Southern States, although some comparisons are made with mortality among northern Negroes and southern whites. Much of the material is presented in graphic form, and detailed tables have been included in the appendix. The bulletin includes a consideration of such questions as the course of mortality since 1920 from all causes and from specific causes, mortality among males and females and in urban and rural areas, the major causes of death among Negroes, the relative importance of specific causes of death among Negroes as compared with whites, and age-specific mortality for detailed causes of death.

Mortality from all causes for all ages declined during the years 1922 to 1932 a total of 2.5 percent among the colored and 7.7 percent among the white population. Each age group under 30 among the colored and under 45 years among the white showed a decline. Over those ages the recorded mortality increased for both races, the percentage increase being more for the colored than for the white.

On the whole the changes in mortality from specific causes have been in the same direction, and for many of the specific causes the change has been at approximately the same rate for both races. The principal causes for which the mortality rates have declined are the acute infectious diseases, respiratory tuberculosis, stomach diseases, and diseases of early infancy. Cancer, arteriosclerosis, and chronic heart diseases have been increasing at about the same rates for colored and white, but acute heart disease and cerebral hemorrhage have increased faster among the colored. The recorded mortality from syphilis has increased more rapidly among colored, and the recorded mortality from locomotor ataxia and general paralysis of the insane has decreased for white and remained about stationary for colored.

Ratios of colored to white death rates at specific ages show that the largest relative differences between colored and white mortality occur in the ages from 15 to 54 years.

With the exception of the age group up to 4 years, colored males and females of the same ages show the same rates of mortality. The relative difference between colored and white females is larger than that between colored and white males, particularly at 15 to 64 years of age. Urban mortality is relatively higher for colored than for white, especially during the active working ages.

Age curves of mortality are shown for specific causes. In the age group from 15 to 44 years respiratory tuberculosis and heart disease account for 30 to 40 percent of the total excess of colored over white mortality. The maximum relative difference between colored and white mortality occurs in early adult life; the peak of the relative excess comes at ages 10 to 14 for respiratory tuberculosis, at 20 to 24

for the infectious diseases, nervous diseases, and pneumonia, at 25 to 34 years for cancer, diseases of the heart, and diseases of the arteries, and at 35 to 44 years for digestive diseases and diseases of the kidneys.

ANOPHELES MOSQUITOES FOUND AT 10,500 FEET ELEVA-TION IN GUATEMALA

Dr. Romeo de Leon, of the Guatemala Department of Health, has recently reported the finding of Anopheles mosquitoes at an altitude of 10,500 feet in the Huehuetenango region of the Republic of Guatemala. This discovery was made while Dr. de Leon was on a 2-months' trip through that region in western Guatemala. The greatest altitude at which these mosquitoes had previously been found in the Republic is reported to be 8,500 feet, at which elevation they had been observed in the Quezaltenango region, which is also in the western part of the Republic.

DEATHS DURING WEEK ENDED JUNE 26, 1937

(From the Weekly Health Index, issued by the Bureau of the Census, Department of Commerce)

		Correspond- ing week, 193 6
Data from 86 large cities of the United States: Total deaths.	7, 612	7, 818
Average for 3 prior years. Total deaths, first 25 weeks of year Deaths under 1 year of age.	1 204 021	230, 784 560
Deaths under 1 year of age. Average for 3 prior years. Deaths under 1 year of age, first 25 weeks of year. Data from industrial insurance companies:	529 14, 474	14, 438
Policies in force	69, 933, 379 12, 242	68, 470, 070 11, 653
Death claims per 1,000 policies in force, annual rate	9. 1 10. 7	8. 9 10. 6

PREVALENCE OF DISEASE

No health department, State or local, can effectively prevent or control disease without knowledge of when, where, and under what conditions cases are occurring

UNITED STATES

CURRENT WEEKLY STATE REPORTS

These reports are preliminary, and the figures are subject to change when later returns are received by the State health officers

Cases of certain communicable diseases reported by telegraph by State health officers for weeks ended July 3, 1937, and July 4, 1936

	Diph	theria	Influ	ienza	Ме	nsles		rococcus ngitis
Division and State	Week ended July 3, 1937	Week ended July 4, 1936	Week ended July 3, 1937	Week ended July 4, 1936	Week ended July 3, 1937	Weck ended July 4, 1936	Week ended July 3, 1937	Week ended July 4, 1936
New England States: Maine			2	 -	19 30	169 2	0	1 0
Vermont	1 5 1 4	1 2	i	i	2 288 37 45	58 460 3 78	0 2 0 0	0 1 0 0
Middle Atlantic States: New York 1 New Jersey Pennsylvania	31 10 19	33 3 43	(2) 5 2	21 1	894 393 927	1, 307 262 616	4 0 8	11 1 5
East North Central States: Ohio Indiana Illinois Michigan	20 10 21 17	11 7 29 10	9 2 8 14	6 7 3 1 8	1, 342 271 490 218	197 15 17 29	2 1 1 2 0	1 1 8 7
Wisconsin West North Central States: Minnesota Jown 3 Missouri	2 2 6	5 3	18	 11	53 2 15 80	72 6 8	2 3 0	0 0
North Dakota	3 1 11	2 3 6	1	3	1 6 15	1 5 14	0 0 1 0	1 0 1 0
Delaware Maryland ¹³⁴ District of Columbia Virginia	5 8	5 3 14 4		i	3 79 42 64	7 186 57 89	1 1 1 4	0 7 0 9
West Virginia North Carolina South Carolina Georgia	5 4	4 S 1 3	25 28	3 37	30 134 18	15 15 14	4 3 0 0	2 4 1 2
Florida ¹ East South Central States: Kentucky Tennessee Alabama ¹	1 1 3 5	3 5 5	1 5	1 33 3	28 71 20	7 35 8	0 1 2 4	1 4 4 0
Mississippi 4 West South Central States: Arkansas Louisiana	3 5 6	5 3 1	2 13	6 9	7 3	 11	3 0	0 0
Oklahoma ⁵ Texas ¹	1 20	5 21	5 76	12 63	23 171	7 86	0 3	0

See footnotes at end of table.

Cases of certain communicable diseases reported by telegraph by State health officers for weeks ended July 3, 1937, and July 4, 1936—Continued

	Diph	theria	Infl	lenza	Me	asles		gococcus ingitis
Division and State	Week ended July 3, 1937	Week ended July 4, 1936	Week ended July 3, 1937	Week ended July 4, 1936	Week ended July 3, 1937	Week ended July 4, 1936	Week ended July 3, 1937	Week ended July 4, 1936
Mountain States: Montana Idaho ³ Wyoming ³ Colorado New Mexico Arizona Utah ⁴ Pacific States:	10	1 1 2 4 3 1	2	3 25	49 23 14 58	1 10 18 27 36	0 0 0 0 0	0 0 0 0 0 0
Washington Oregon 3 California	1 4 25	1 24	10 14	4 444	72 9 97	97 14 1, 467	1 0 6	1 0 4
Total	290	287	259	686	6, 225	5, 642	61	78
First 26 weeks of year	11, 649	12, 685	272, 835	138, 583	224, 733	256, 289	3, 709	5, 408
	Polion	nyelitis	Scarlet	fever	Sma	llpox	Typho	id fever
Division and State	Week ended July 3, 1937	Week ended July 4, 1936	Week ended July 3, 1937	Week ended July 4, 1936	Week ended July 3, 1937	Week ended July 4, 1936	Week ended July 3, 1937	Week ended July 4, 1935
New England States: Maine New Hampshire Vermont Massachusetts Rhode Island Connecticut Middle Atlantic States: New York 1 New York 1 New Jersey Pennsylvania East North Central States: Ohio	1 0 0 2 0 0	0 0 0 0 0 0 0	11 6 5 122 10 37 235 57 284	6 1 15 74 8 14 293 80 379	0 0 0 0 0 0	0 0 0 0 0 0	2 1 0 1 0 0 1 1 1 17	0 0 0 2 0 0 0 8 3 14
Indiana	0 2 1 0	1 2 0 0	183 337 113	27 235 204 136	9 28 0 1	1 17 0 9	3 8 4 0	0 6 5 2
Minnesota	0 0 1 0 0 1 2	0 0 1 0 0 0	49 52 35 5 11 8	61 41 26 3 15 16 51	7 20 10 12 4 2	13 7 6 3 4 10 2	0 3 31 0 0 0	2 2 6 0 0 0
Bouth Atlantic States: Delaware Maryland 124 District of Columbia Virginia West Virginia North Carolina 1 South Carolina 1 Florida 1 Florida 1 East South Central States: Kentucky Tennessee	0 0 0 1 1 7 1 4 0	0 0 0 1 1 0 0 3 1	2 14 4 1 18 14 1 10 7	24 9 13 20 5 2 4 2	0 0 0 0 0 0 0	0 0 0 0 1 0 0 0	2 9 7 6 7 30 20 50 4	0 2 0 1 2 2 6 9 22 0
Alabama 1	30	36	5	7 4	. 0	ŏ	10 17	10 15
Arkansas Louisiana Oklahoma ⁵ Texas ¹	26 7 7 23	0 0 1 0	9 8 7 40	5 10 13	0 0 3 1	0	29 19 7 35	20 11 35

See footnotes at end of table.

Cases of certain communicable diseases reported by telegraph by State health officers for weeks ended July 3, 1937, and July 4, 1936—Continued

31	Poliomyelitis		Scarle	fever	Sma	llpox	Typho	id fever
Division and State	Week ended July 3, 1937	Week ended July 4, 1936	Week ended July 3, 1937	Week ended July 4, 1936	Week ended July 3, 1937	Week ended July 4, 1936	Week ended July 3, 1937	Week ended July 4, 1936
Mountain States: Montana Idaho ³ W yoming ³ Colorado New Mexico Arizona Utah ⁴ Pacific States: W ashington Oregon ³ California	0 1 0 1 0 1 0 1	0 0 0 0 0 0 0 0	9 11 12 10 5 1 11 11 13 12 93	14 2 11 26 23 2 19 14 7	11 77 0 2 2 0 1	19 2 0 2 0 0 8 3 2 3	1 2 1 2 2 4 0	2 3 0 0 7 3 3 14 9 5
Total	158	61	2, 139	2, 201	151	112	421	280
First 26 weeks of year	815	523	157, 273	170, 843	7, 370	5, 522	3, 791	3, 710

SUMMARY OF MONTHLY REPORTS FROM STATES

The following summary of cases reported monthly by States is published weekly and covers only those States from which reports are received during the current week:

State	Menin- gococ- cus menin- gitis	Diph- theria	Influ- enza	Mala- ria	Mea- sles	Pel- lugra	Polio- mye- litis	Scarlet fever	Small- pox	Ty- phoid fever
May 1937										
Florida Montana	7	31 2	22 38	54	118 6 5		2	34 73	0 64	12 2
North Dakota	2	2 8	99	3	5 43		0 3	122 186	76 50	5 9
Wisconsin	1	17	113		263		1	1,092	8	6

May 1937		May 1937—Continued	,	May 1937—Continued	
Chicken pox: Ca	ses	Mumps—Continued	Cases	Trachoma:	Cascs
Florida	119	North Dakota	42	Montana	2
	121	Oregon		Trichinosis:	
North Dakota	81	Wisconsin	722	_ Florida	15
	216	Paratyphoid fever:	_	Tularaemia:	_
Wisconsin 2,	590	Florida	5	Montana	. 3
Dysentery:	_	Rabies in animals:		Typhus fever:	
Florida (bacillary)	2	Oregon	4	Florida	. 4
Wisconsin (amoebic)	1	Rocky Mountain spotted		Undulant fever:	
Encephalitis, epidemic or		fever:		Wisconsin	. 9
lethargic:	_	Montana	6	Vincent's infection:	_
Wisconsin	1	North Dakota	1	Florida	
German measles:		Oregon	16	Montana	
	122	Scabies:		North Dakota	
Hookworm disease:		Montana	1	Oregon	10
	663	Oregon	21	Whooping cough:	
Impetigo contagiosa:		Septic sore throat:		Florida	
Montana	4	Montana	17	Montana	
Oregon	32	Oregon	7	North Dakota	
Mumps:		Wisconsin	10	Oregon	116
	120	Tick paralysis:		Wisconsin	458
Montana	259	Montana	1		

¹ Typhus fever, week ended July 3, 1937, 59 cases, as follows: New York, 2; Maryland, 2; Georgia, 17; Florida, 7; Alabama, 24; Texas, 7.

1 New York City only.

Rocky Mountain spotted fever, week ended July 3, 1937, 14 cases, as follows: Iowa, 2; Maryland, 4; North Carolina, 1; Idaho, 4; Wyoming, 1; Oregon, 2.

Wesk ended earlier than Saturday.

Figures for 1936 are exclusive of Oklahoma City and Tulsa.

Two nonparalytic cases included.

Two nonparalytic cases included.

984

WEEKLY REPORTS FROM CITIES

City reports for week ended June 26, 1937

This table summarizes the reports received weekly from a selected list of 140 cities for the purpose of showing a cross section of the current urban incidence of the communicable diseases listed in the table. Weekly reports are received from about 700 cities, from which the data are tabulated and filed for reference.

State and city	Diph- theria	1	uenza	Mea- sles	Pneu- monia	Scar- let	Small- pox	Tuber- culosis	Ty- phoid	Whoop- ing	Deaths,
	cases	1	Deaths	cases	deaths	fever cases	cases	deaths	fever cases	cases	causes
Data for 90 cities:											-:1)
5-year average Current week 1	171 126	58 20	21 16	3, 637 2, 594	394 353	1, 134 945	11 12	395 397	54 43	1, 468 1, 334	<i>X</i>
Maine:			0								
Portland New Hampshire:	0		-	0	1	2	0	1	0	2	20
Concord Manchester	0		0	0	0	0	0	0	0	0	: 3 11
Nashua	ŏ		ŏ	ŏ	ŏ	ĭ	ŏ	ĭ	ŏ	ŏ	6
Vermont: Barre	0		0	0	0	0	0	0	0	0	3
Burlington	1		0	0	0	0	0	0	0	0	5 7
Rutland						- 1	- 1			0	
Boston	1		0	27 51	14 0	37	0	16 1	1 0	35 2	. 185 20
Fall River Springfield	0		0	0	1	5	0	4	1	5	36
Worcester Rhode Island:	2		0	7	3	4	0	5	0	4	
Pawtucket	1		0	0	0	0	0	0	0	0	11
Providence	0		0	34	2	20	0	1	0	39	50
Bridgeport	0		0	.0	0	22	0	o l	o l	0	22
Hartford New Haven	0		0	13 2	1	3	0	0	0	5 1	34 36
lew York:			ĺ		- 1	1	- 1	-		ļ	
Buffalo New York	0 38	2	0	37 536	9 65	99	0	5 83	0 10	17 81	120 1, 278
Rochester	0		0	11	2	9	0	0	0	14	57
Syracuselew Jersey:	0		0	19	3	4	0	0	0	27	34
Camden	0	1	1	3	o l	2	0	0	0	1	26
Newark Trenton	0		1 0	10 32	8 0	8 2	0	5 6	0	10	82 26
ennsylvania:	2		1		- 1	- [- 1		
Philadelphia Pittsburgh	2 0		0	28 253	14 16	61 26	0	19 6	6 3	63 49	393 177
Reading	0		ō	18	ĭ	3 5	0	i	0	3 2	21
Scranton	۰ı			1		٥	0		٩	2	
hio: Cincinnati	0	1	6	12	6	10	0	12	0	34	129
Cleveland	5 0	1	0	369 21	15	28	8	13	2 0	37 15	174
Columbus Toledo	4	2	1 1	184	0 2	2 2	ŏ	9	ŏ	38	63 68
ndiana:	0	1	0	21	0	1	0	0	اه	2	11
Fort Wayne				.	-				-		
Indianapolis South Bend	0		1	99	6	5	0	5	0	34	114 14
Terre Haute	Ŏ.		ô	ĭ	ô	î	î	õ	ŏ	ŏ	19
linois:	0		0	اه	1	1	o	0	o	اه	7
Chicago	26 0	1	0	406	27	120	0	48	οl	62	691
Elgin Moline	ŏ l		0	0	0	0	0	0	0	0	· / 6 13
Springfield	0		ŏ	5	ŏ	ĭ	ō	ŏ	0	6	33
lichigan: Detroit	9 .		o	110	9	238	0	17	1	38	282
FlintGrand Rapids	0		Ŏ	2 21	4	4	0	1 0	0	17	26 27
isconsin:			- 1	- 1		8	0	- 1	1		
Kenosha	8 -		0	1 1	0	6	0	0	0	0 10	8 23
WI 4013011	ŏŀ		٧١		2	a± 1		Ņ,	ĭ		113
Madison Milwaukee Racine	ŏ		0	11 0	7 0	35 2	0	0	ő	20	7

¹ Figures for Fort Wayne, Ind., estimated; report not received.

City reports for week ended June 26, 1937—Continued

State and city	Diph- theria		uenza	Mea- sles	Pneu- monia	Scar- let fever	Small- pox	Tuber- culosis	Ty- phoid fever	Whoop- ing cough	Deaths, all causes
	cases	Cases	Deaths	cases	deaths	cases	cases	deaths	cases	cases	
Minnesota:											
Duluth	0		0	0	1	13	Ó	4	Q	1	25
Minneapolis	1 0		0	0	2 5	16 0	0	0	0	72	64 63
St. Paul Iowa:	١		١	٠ ا	"	١	۰	1	U	1 12	[∞]
Cedar Rapids	0	 		1		1	0		0	1	
Des Moines	0		0	1	0	4	3	0	0	Õ	27
Sioux City Waterloo	1 0		0	0	0	3 5	0	0	0	5	0
Missouri:	1			ľ			1			ľ	
Kansas City	0		0	5	6	11	0	3	0	6	100
St. Joseph St. Louis	0 3		0	0 48	2 6	35	0	1 9	0	37	24 186
North Dakota:	l		1		1						İ
Fargo	0		0	0	0	0	0	0	0	19	8
Grand Forks	0		0	0	0	0	0		0	111	
MinotSouth Dakota:	٥			1	١ ١	۰		ا ا	·	١ ،	
Aberdeen	0			0		0	0		Ō	0	
Sioux Falls	0		0	0	0	0	0	0	0	0	9
Nebraska: Omaha	0		0	2	1	o	7	0	0	7	50
Kansas:					i 1						ł
Lawrence	0		0	0	0	1	0	0	0	.2	5
Topeka	0		0	1 8	2 2	3 2	0	0 2	0	12 14	13 37
Wichita	·		١		~	-		-	•	**	٠.
Delaware:	_			_	_						
Wilmington	0		0	0	2	1	0	1	0	0	21
Maryland: Baltimore	4		0	66	11	6	0	15	1	81	184
Cumberland	Ô		ŏ	0	1	1	0	0	0	13	14
Frederick	0		0	0	0	0	0	0	0	0	5
Dist. of Col.: Washington	7		0	43	7	9	o	16	3	13	157
Virginia:	•		١	10	٠,	١	١	•	١	10	101
Lynchburg	0		0	3	0	0	0	1	0	9	7
Norfolk Richmond	0			11	2 0	0	0	0	0	3 0	26 44
Ronnoud	0		0	0	0	0	ŏ	ō	ŏ	6	13
West Virginia:			f			- 1		i			
Charleston	0		0	0	2	0	0	1	0	4	28
Huntington Wheeling	1		0	0 7	0	$\frac{1}{7}$	0		0	14	12
North Carolina:	۰		١		, , ,	• 1	1	-			
Gastonia	0			0		0	0		0	3	
Raleigh	0		0	1 0	0	0	0	$\begin{bmatrix} 0 \\ 2 \end{bmatrix}$	0	2 0	12 13
Wilmington Winston-Salem		0	ŏ	3	2	2	ŏ	ő	ŏl	13	16
South Carolina:		١	- 1	-		l			.	- 1	
Charleston	0		0	2	1	1	0	1	1 0	2	22 8
Columbia Florence	0		8	0	1 0	0	0	0	ő	1	14
Greenville	ŏ		ŏ	ŏ	ĭ	ŏ	ŏ	ô	ŏ	4	5
Georgia:				ا ہ		. 1	ا م		ا		- 00
Atlanta Brunswick	0	3	0	0	1 0	0	0	0	2	12 13	90 4
Savannah	ŏ	2	ŏ	ŏl	3	ŏ	ŏ	ĭ	ŏ	ő	28
Florida:					.	_	_	_		_	
Miami Tampa	0	1	0	0 5	1 0	0	0	3 1	1 0	0 2	31 22
-		i			I	l		- 1		1	
Kentucky: Ashland	1		- 1	123		1	0		2	19	
Covington	δl	1	i	10	1	ő	0	0	0	11	11
Lexington	0		0	5	0	0	o l	0	0	8	20
Louisville	0	3	1	46	6	6	0	3	0	48	86
Fennessee: Knoxville	0		0	0	1	1	0	0	1	5	28
Memphis	0		1	27	1	0	0	Ō	2	32	63
Nashville	0		0	2	3	0	0	4	0	13	51
Uabama: Birmingham	اه	i	0	19	4	0	0	4	0	4	76
Mobile	ŏ		ŏ	0	i	ŏ	ŏ	i	0	0	26
Montgomery	Ö			0		0	0		0	0	
l who moose		1	l	1	- 1	- 1	1	- 1	- 1	ı	
Arkansas: Fort Smith	0			1		3	0 .		0	1].	
Little Rock	ŏΙ		0	1 0	3	ōŀ	Ŏ l	i	0	0	5

City reports for week ended June 26, 1937—Continued

	Diph-	Inf	luenza	Mea-	Pneu-	Scar- let	Small		Ty- phoid	Whoop-ing	Deaths,
State and city	theria cases	Cases	Deaths	sles cases	monia deaths	fever cases	pox cases	culosis deaths	fover	cough	all causes
				ļ	ļ						
Louisiana: Lake Charles	0			0							1
New Orleans	9	2	i	1	11	4	0	7	2	26	141
Shreveport Oklahoma:	0		0	0	3	0	0	3	3	0	50
MuskogeeOklahoma City	0		0	0	4	1 2	0	3	0	0	1:
Tulsa	0			10		ő	ŏ		ĭ	48	
Texas:	1		0	12	2	2	0	2	0	16	73
Dallas Fort Worth	Õ		Ō	2	0	Ō	Ó	Ō	0	i	25
Galveston Houston	0		0	0	0	0 2	0	0	0	0	9 94
San Antonio	ŏ		ŏ	ŏ	4	ō	Ŏ	13	Ŏ	7	82
Montana:									1		
Billings Great Falls	0		0	0	1	6 2	0	0	0	0	. 2 9
Helena	Ó		0	1	0	0	0	Ó	0	3	3
Missoula Idaho:	0		0	0	0	0	2	0	0	0	4
Boise	0		0	1	1	0	0	0	0	0	6
Colorado: Colorado Springs_	0		0	0	o	0	0	1	0	0	13
Denver	0		0	42	5	5	0	3	1	17	78
Pueblo New Mexico:	1		0	0	3	1	0	1	0	0	14
Albuquerque	0		0	1	1	1	0	1	0	2	10
Utah: Salt Lake City	0		0	61	1	7	0	0	0	23	22
Washington:			_							_	***
Seattle Spokane	0		0	24 31	3 1	3	0	0	0	7 22	73 19
Tacoma	Ó		0	0	0	2	0	2	0	3	24
Oregon: Portland	1		0	2	2	8	1	2	0	7	65
SalemCalifornia:	0	1		0		1	0		0	3	
Los Angeles	5	3	2	5	16	18	0	22	1 1	99 12	297 23
Sacramento San Francisco	3	3-	0	10 24	1	7	8	7	0	62	148
	1		T I					1.	\f !		
	Me	ningoco neningi	tis F	Polio-				1.	Mening menir		Polio-
State and city			1	nve- li t is	:	State a	nd city	-			mye- litis
	Cas	ses D		eases				İ	Cases	Deaths	cases
	_	_									
Massachusetts:			ļ		Distri	ict of C	clumbi	a:		ł	
Boston Springfield		2	0	0	Virgir	ashing	ton		1	0	0
Rhode Island:	1	ı		-	Nor	folk			1	2	0
Providence New York:		1	0	0	Georg	ia: tlanta_			0	0	1
Buffalo		1	0	0	Florid	la:			ľ	- 1	
New York Pennsylvania:		4	0	2	Tenne	liami essee:			1	°	1
Philadelphia		0	0	1	K	nexvill	e		1 0	8	0 4
Ohio: Cincinnati		1	0	3	l N	asnviii	S O		ŏ	ŏ	2
Cleveland		1	0	0	Alaba		ha m		3	1	. 0
Indianapolis		0	1	0	Leuisi	ana:		1	- 1	ı	
Illinois: Chicago	_	1	0	0	Texas		eans		1	0	1
Wisconsin:		0	0	1	H	custon.	 -		1	0	4
Milwaukee	-	- 1		- 1		os Ange	eles		2	4	o
St. Louis	-	1	0	0	Sa	n Fran	cisco		0	0	1
Baltimore	_	1	1	0	1			L			

Encephalitis, epidemic or lethargic.—Cases: New York, 4.

Pellagra.—Cases: Chicago. 1; Wichita, 1; Winston-Salem, 1; Charleston, S. C., 2; Atlanta, 1; Savannah, 4; Mobile, 1; New Orleans, 6; Los Angeles, 1.

Rabies in man.—Deaths: Los Angeles, 1.

Typhus fever.—Cases: Galveston, 1; Houston, 1. Deaths: Galveston, 1.

FOREIGN AND INSULAR

CANADA

Provinces—Communicable diseases—2 weeks ended June 19, 1937.—During the 2 weeks ended June 19, 1937, cases of certain communicable diseases were reported by the Department of Pensions and National Health of Canada, as follows:

Cerebrospinal meningitis. 4 2 2 1 1 Chicken pox. 7 12 210 585 33 148 15 Diphtheria. 3 2 2 51 15 5 3 1 Dysentery. 5 1 <td< th=""><th>Mani- toba Sas- katch- toba Ras- katch- ta Brit- ish Colum- bia To</th><th>Onta- rio</th><th>Que- bec</th><th>New Bruns- wick</th><th>Nova Scotia</th><th>Prince Ed- ward Island</th><th>Disease</th></td<>	Mani- toba Sas- katch- toba Ras- katch- ta Brit- ish Colum- bia To	Onta- rio	Que- bec	New Bruns- wick	Nova Scotia	Prince Ed- ward Island	Disease
Erysipelas	5 33 148 15 123 1	585	210 51		7 2	3	Chicken pox
Mumps 4 14 346 4 1 9	3 232 196 233 278 3	1, 523	6 1 538	5 14	127	3	Influenza Measles
Paratyphoid fever 1 Pneumonia 38 Poliomyelitis 7 Scarlet fever 1 27 2 161 268 29 45 123	i	1		2	27	6	Paratyphoid fever Pneumonia Poliomyelitis Scarlet fever
Trachoma 4 20 25 159 140 24 24 3 Typhoid fever 1 5 30 3 2 2 2 Undulant fever 3 3 2 2 2 Whooping cough 1 316 246 166 8 11	3 2 2 1	3 8	30 3		20 1	4	Tuberculosis Typhoid fever Undulant fever

CZECHOSLOVAKIA

Communicable diseases—April 1937.—During the month of April 1937, certain communicable diseases were reported in Czechoslovakia as follows:

Disease	Cases	Deaths	Disease	Cases	Deaths
Anthrax Cerebrospinal meningitis Chicken pox Diphtheria Dysentery Influenza Malaria Paratyphoid fever	3 20 225 1, 793 13 98 373 10	97 8	Poliomyelitis. Puerperal fever. Scarlet fever. Trachoma. Tularaemia. Typhoid fever. Typhus fever.	3 31 1,867 64 30 324 25	1 6 34 22

FINLAND

Communicable diseases—May 1937.—During the month of May 1937, cases of certain communicable diseases were reported in Finland as follows:

Disease	Cases	Disease	Casas
Diphtheria		PoliomyelitisScarlet fever	8 1, 235
DysenteryInfluenza		Typhoid fever	

ITALY

Communicable diseases—4 weeks ended April 25, 1937.—During the 4 weeks ended April 25, 1937, cases of certain communicable diseases were reported in Italy as follows:

	Mar. 2	9-Apr. 4	Apr	. 5–11	Apr.	12-18	Apr.	19–25
Disease	Cases	Com- munes affected	Cases	Com- munes affected	Cases	Com- munes affected	Cases	Com- munes affected
Anthrax Cerebrospinal meningitis Chicken pox Diphtheria Dysentery Hookworn disease Lethargic encephalitis Measles Mumps Paratyphoid fever Poliomyelitis Puerperal fever Scarlet fever Typhoid fever Undulant fever Whooping cough	4 30 483 462 5 4 2 2,076 528 27 25 37 312 200 98	4 25 162 228 5 3 2 323 133 222 23 32 128 147 59 155	77 23 470 466 3 9 4 1,868 575 24 17 37 382 198 128 655	7 19 177 243 3 5 4 345 122 22 12 22 12 36 130 129 89 207	12 21 381 468 7 21 3 1, 786 599 36 19 30 395 224 133 597	12 18 147 225 7 4 3 338 141 32 18 30 125 143 81 187	8 26 479 465 5 11 1,640 418 42 24 28 336 224 109 519	6 23 172 231 5 5 1 337 124 31 222 26 118 149 75 5 169

CHOLERA, PLAGUE, SMALLPOX, TYPHUS FEVER, AND YELLOW FEVER

NOTE.—A table giving current information of the world prevalence of quarantinable diseases appeared in the Public Health Reports for June 25, 1937, pages 858-871. A similar cumulative table will appear in the Public Health Reports to be issued July 30, 1937, and thereafter, at least for the time being, in the issue published on the last Friday of each month.

Cholera

Straits Settlements—Penang.—During the week ended June 19, 1937, 1 case of cholera was reported in Penang, Straits Settlements.

Smallpox

Mexico.—During the month of March 1937, smallpox was reported in Mexico as follows: Mexico State, 1 case; Mexico, D. F., 16 cases, 4 deaths; Queretaro State, 3 cases.

Typhus Fever ·

Mexico.—During the month of March 1937, typhus fever has been reported in Mexico as follows: Aguascalientes, Aguascalientes State, 1 case; Guadalajara, Jalisco State, 1 death; Guanajuato, Guanajuato State, 2 cases, 1 death; Mexico State, 1 case, 1 death; Mexico, D. F., 12 cases, 6 deaths; Queretaro State, 1 case, 1 death; San Luis Potosi, San Luis Potosi State, 2 cases.

Yellow Fever

Gold Coast.—Yellow fever has been reported in Gold Coast as follows: June 24, 1937, Accra, 1 fatal case; Mepom, 1 fatal case. June 30, 1937, Adeiso, 1 fatal case; Huhunya, 1 fatal case; Swedru, 1 case. Senegal—Eambey.—On June 25, 1937, 1 suspected case of yellow fever was reported in Bambey, Senegal.